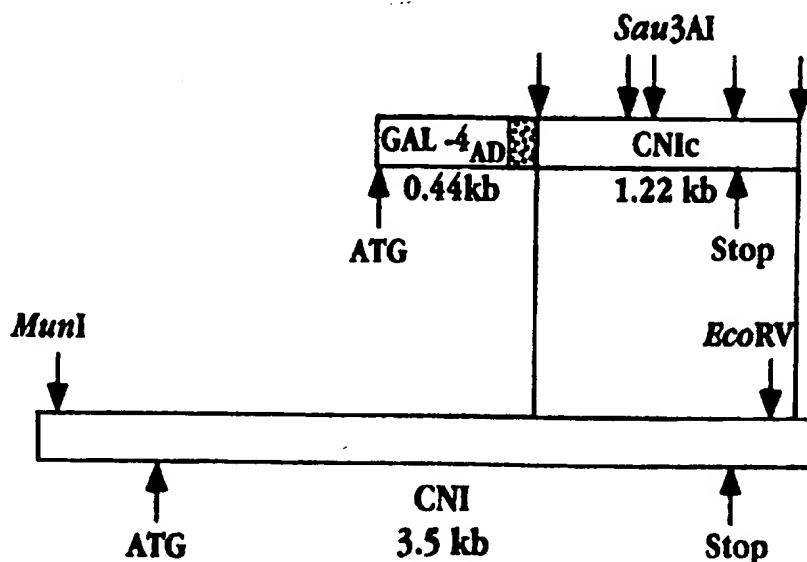


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<b>(21) International Application Number:</b> PCT/US95/13580 <b>(22) International Filing Date:</b> 23 October 1995 (23.10.95)  <b>(30) Priority Data:</b> 08/328,322                      24 October 1994 (24.10.94)                      US  <b>(71) Applicant:</b> THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY [US/US]; Stanford, CA 94305 (US).  <b>(72) Inventors:</b> HUANG, Laiqiang; 365 Chiquita Avenue, #8, Mountain View, CA 94041 (US). CYERT, Martha, S.; 590 Ashton Avenue, Palo Alto, CA 94306 (US).  <b>(74) Agent:</b> SHOLTZ, Charles, K.; Dehlinger & Associates, P.O. Box 60850, Palo Alto, CA 94306-1546 (US).	<b>(81) Designated States:</b> AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published.</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

**(54) Title:** CALCINEURIN INTERACTING PROTEIN COMPOSITIONS AND METHODS**(57) Abstract**

An identification and characterization of a calcineurin interacting (CNI) protein effective to enhance immunosuppressive effects of calcineurin-targeted immunosuppressants by potentiating an interaction of an immunophilin with calcineurin is described herein. One embodiment of the invention is the CNI polypeptide encoded by the *CNI* gene of *Saccharomyces cerevisiae*. Polynucleotides encoding a CNI protein are also described. Also described are yeast cells carrying mutations in the *CNI* gene. Further, a method of identifying a small molecule immunosuppressant compound is described. The methods include the use of a cell-based two hybrid protein-protein interaction assay, wherein one of two fusion hybrid proteins in a cell contains a subunit of calcineurin, and the other of two fusion hybrid proteins contains an immunophilin.



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**CALCINEURIN INTERACTING PROTEIN**  
**COMPOSITIONS AND METHODS**

**Field of the Invention**

- 5       The present invention relates to compounds affecting the function of calcineurin, particularly interactions of calcineurin with immunosuppressant drugs.

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### 30 Background of the Invention

The immune system functions as the body's major defense against diseases caused by invading organisms. This complex system fights disease by killing invaders such as bacteria, viruses, parasites or cancerous cells while leaving the body's normal tissues unharmed. The immune system's ability to distinguish the body's normal tissues, or self, from foreign or

cancerous tissue, or non-self, is an essential feature of normal immune system function. A second essential feature is memory, the ability to remember a particular foreign invader and to mount an enhanced defensive response when the previously encountered invader returns. The loss of recognition of a particular tissue as self and the subsequent immune response directed against that tissue produce serious illness.

An autoimmune disease results from the immune system attacking the body's own organs or tissues, producing a clinical condition associated with the destruction of that tissue. An autoimmune attack directed against the joint lining tissue results in rheumatoid arthritis; an attack against the conducting fibers of the nervous system results in multiple sclerosis. The autoimmune diseases most likely share a common pathogenesis and the need for safe and effective therapy. One type of therapy that has been employed in combating autoimmune disease is treatment with immunosuppressant drugs, such as cyclosporin A, FK506 and rapamycin. While the treatments are often effective, the drugs typically have undesirable side effects, including neurotoxicity, nephrotoxicity, hypertension, and metabolic disorder. Many of these side effects are due to the drugs' action on cells other than those of the immune system.

In addition to their use in treating autoimmune conditions, immunosuppressive agents have also been used in treating or preventing transplantation rejection. Organ transplantation involving human organ donors and human recipients (allogeneic grafts), and non-human primate donors and human recipients (xenogeneic grafts), has received considerable medical and scientific attention (*e.g.*, Roberts, 1989; Platt, 1990). To a great extent, this effort has been aimed at eliminating, or at least reducing, the problem of rejection of the transplanted organ. In the absence of adequate immunosuppressive therapy, the transplanted organ is destroyed by the host immune system.

Presently, the most commonly used agents for preventing transplant rejection include corticosteroids, cytotoxic drugs that specifically inhibit T cell activation such as azathioprine, immunosuppressive drugs such as cyclosporin A, and specific antibodies directed against T lymphocytes or surface receptors that mediate their activation (Briggs, 1991; Kennedy, 1983; Storb, 1985; Storb, 1986). All of these drug therapies are limited in effectiveness, in part because the doses needed for effective treatment of transplant rejection may increase the patient's susceptibility to infection by a variety of opportunistic invaders, and in part because of direct toxicity and other side effects.

Cyclosporin A, currently the most effective and most commonly used agent, is significantly toxic to the kidney. This nephrotoxicity limits the quantity of drug that can be

safely given. The physician is frequently forced to administer sub-optimal doses of the drug because of this toxicity. A preparation capable of potentiating the action of immunosuppressive agents such as cyclosporin A on the immune system, thus allowing the administration of lower doses of drug, would be of considerable value in reducing the morbidity and mortality associated with transplantation.

### Summary of the Invention

In one embodiment, the present invention includes polypeptide compositions effective to enhance immunosuppressive effects of calcineurin-targeted immunosuppressants by potentiating an interaction of an immunophilin with calcineurin. The present invention includes the isolation and characterization of a calcineurin interacting protein, CNI, having these properties. Also disclosed herein are methods for the isolation and characterization of further CNI-related sequences and sequences of CNI-variants. The amino acid sequences presented as SEQ ID NO:2 and SEQ ID NO:5 are exemplary of the polypeptides of the present invention.

The present invention also includes a CNI polypeptide fragment that interacts specifically with the "A" subunits of calcineurin (CNA1 and CNA2), but not with calcineurin "B" subunit (CNB1). In one embodiment, this fragment has an amino acid sequence of between 15 and 915 amino acids in length, for example, the c-terminal 306 amino acids of the CNI protein (CN1c).

Included aspects of the invention are an CNI polypeptide; a recombinant CNI polypeptide; and a fusion polypeptide comprised of an CNI polypeptide. Exemplary fusion proteins include fusions to  $\beta$ -galactosidase.

The invention further includes isolated nucleic acid sequences encoding the above described polypeptides and polypeptide fragments. Exemplary nucleic acid sequences include the sequences presented as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:6. The present invention includes CNI-encoding genomic polynucleotides, cDNAs thereto and complements thereof. With respect to polynucleotides, some aspects of the invention include: a purified CNI-encoding genomic polynucleotide; CNI polypeptide-encoding RNA and DNA polynucleotides; recombinant CNI polypeptide-encoding polynucleotides; a recombinant vector including any of the above recombinant polynucleotides, and a host cell transformed with any of these vectors. Another aspect of the invention is a polynucleotide probe for CNI polypeptide-encoding sequences.

Portions of a CNI-polypeptide coding sequences are effective as probes to isolate variants coding sequences which occur naturally, or to determine the presence of such coding

sequences in nucleic acid samples. Such probes include hybridization screening probes and polymerase chain reaction amplification primers specific for CNI-polypeptide coding sequences. Homologues of CNI may be isolated from a number of sources, such as other types of yeast cells (*e.g.*, *Schizosaccharomyces*) or mammalian cells (*e.g.*, human).

5 Other aspects of the invention include: a recombinant expression system which incorporates an open reading frame (ORF) derived from CNI polypeptide-encoding sequences, wherein the ORF is linked operably to a control sequence which is compatible with a desired host, a cell transformed with the recombinant expression system, and a polypeptide produced by the transformed cell. Typically the expression system includes a vector having (a) a nucleic  
10 acid containing an open reading frame that encodes a CNI-polypeptide; and (b) regulatory sequences effective to express said open reading frame in a host cell. The regulatory sequence may include sequences useful for targeting or secretion of the CNI-polypeptide: such as a secretory signal recognized in yeast or bacterial expression systems.

The invention includes a method of recombinantly producing CNI-polypeptides. In the  
15 method, a recombinant expression system containing an open reading frame (ORF) having a polynucleotide sequence which encodes a CNI-polypeptide, where the vector is designed to express the ORF in the host, is introduced into suitable host cells. The host is then cultured under conditions resulting in the expression of the ORF sequence. The CNI-polypeptide sequences discussed above are examples of suitable CNI-polypeptides. Numerous vectors and  
20 their corresponding hosts are useful in the practice of this method of the invention, including, but not limited, to the vectors described herein for expression in yeast cells, and lambda gt11 phage vector and *E. coli* cells. Other host cells include insect and mammalian cell expression systems.

The invention also includes purified antibodies that are immunoreactive with a CNI-  
25 polypeptide. The antibodies may be polyclonal or monoclonal. Antibodies that are specifically immunoreactive with CNI-polypeptides may be useful for the isolation of CNI-polypeptide homologues from other cell type sources (*e.g.*, mammalian).

The present invention also includes, a method of identifying a small molecule immunosuppressant compound. In the method, a cell-based two hybrid protein-protein  
30 interaction assay is constructed where one of two fusion hybrid proteins in the cell contains a subunit of calcineurin, and the other of two fusion hybrid proteins contains an immunophilin. The cell is then contacted with the small molecule being tested. A small molecule is identified as an immunosuppressant if the molecule potentiates an interaction between the two hybrid proteins. In one embodiment, the method is carried out using yeast cells, where one of the two

fusion hybrid proteins contains a GAL4 activation domain and the other of two fusion hybrid proteins contains a GAL4 binding domain. The method can be carried out where subunits of calcineurin are from any cell source, in particular, yeast or mammalian cells (including human cells). The subunit may, for example, be yeast calcineurin subunit CNA1 or CNA2, or human  
5 calcineurin subunit "A". The immunophilin can, for example, be cyclophilins or FK506-binding proteins (*e.g.*, FKBP12) typically from a homologous cell source.

Also included in the present invention is another method of identifying a small molecule immunosuppressant compound. In the method, a cell-based two hybrid protein-protein interaction assay is constructed, wherein one of two fusion hybrid proteins in a cell contains  
10 an "A" subunit of calcineurin, and the other of two fusion hybrid proteins contains a CNI polypeptide. The cell preferably, but not necessarily, also contains a vector construct causing overexpression, or increased expression, of a "B" subunit of calcineurin. The cell is then contacted with the small molecule being tested. A small molecule is identified as an immunosuppressant if the molecule potentiates an interaction between the two hybrid proteins.  
15 This method is used to identify compounds (like FK506) that potentiate the interaction between CNI and CNA1. In one embodiment, the method is carried out using yeast cells, where one of the two fusion hybrid proteins contains a GAL4 activation domain and the other of two fusion hybrid proteins contains a GAL4 binding domain. The method can be carried out where subunits of calcineurin are from any cell source, in particular, yeast or mammalian cells  
20 (including human cells). The subunits may, for example, be calcineurin subunit A1 or A2. The CNI polypeptide may also be from any source (*e.g.*, yeast or human), and may be only a fragment of a complete CNI polypeptide (such as a c-terminal fragment). An exemplary c-terminal fragment of CNI is CN1c.

Further, included in the present invention, is a yeast cell carrying a mutation in the  
25 naturally-occurring copy of *CNI*, where the mutation prevents expression of a functional CNI protein from the genomic copy. Embodiments of this aspect of the present invention include deletion mutations within the coding region of the *CNI* gene, deletion of regulation regions of the *CNI* gene, and non-sense or mis-sense mutations in the *CNI* gene. Yeast cells having such mutations are useful, for example, in a method of identifying proteins of similar function to  
30 CNI. In one embodiment, a hybrid interaction screen is set up in a cell with a CNI deletion and a GAL4 protein binding domain-CNA fusion and a GAL4 activation domain-immunophilin fusion. Expression libraries are then screened to identify clones encoding proteins that potentiate an interaction of an immunophilin with calcineurin. This screen will identify CNI-coding sequences as well as other proteins with a similar function.



In a related embodiment, a yeast cell with a CNI deletion is used to identify CNI homologues (*e.g.*, from other organisms, such as human) using a complementation assay or screen. Expression libraries (*e.g.*, human lymphocyte expression libraries) are transformed into cells with a CNI deletion, and transformants are selected on their ability to complement the function of yeast CNI. An exemplary assay for selecting such transformants is exposure to hygromycin B. Cells which become more sensitive to hygromycin B following transformation are further analyzed to determine if the plasmid with which they were transformed contains an insert homologous to yeast CNI, or encoding a polypeptide with similar function to CNI.

The invention also includes a yeast cell carrying a mutation in the naturally-occurring genomic copy of a gene encoding calcineurin-interacting polypeptide, where the mutation prevents expression of a functional calcineurin-interacting polypeptide from the genomic copy. The mutation may be a null mutation, such as described in Example 8 below, or a different type of mutation, *e.g.*, a nonsense or missense mutation. Nonsense and missense mutations may be generated using standard methods.

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

#### **Brief Description of the Figures**

Figures 1A and 1B present schematic diagrams of sequences encoding the c-terminal portion of CNI (CN1c) fused to GAL-4 activation domain (GAL-4AD) (Fig. 1A), and sequences encoding CNI (Fig. 1B).

Figure 2A presents data from a  $\beta$ -galactosidase ( $\beta$ -gal) assay to detect the interaction of CN1c with the A1 subunit of calcineurin (CNA1), A2 subunit of calcineurin (CNA2), GAL-4 binding domain (G4BD) and lamin C. A labeled schematic diagram corresponding to the data shown in Fig. 2A is presented in Fig. 2B to facilitate reference to individual groups of colonies.

Figure 3A presents data from a  $\beta$ -gal assay to detect the interaction of CN1c with CNA1 $\Delta$ C, CNA2 $\Delta$ C and CNB1. A labeled schematic diagram corresponding to the data shown in Fig. 3A is presented in Fig. 3B.

Figures 4A and 4C present data from  $\beta$ -gal assays to evaluate the effects of FK506 and the deletion of CNB1 on the interactions of CN1c with CNA and CNA $\Delta$ C. Labeled schematic diagrams corresponding to the data shown in Figs. 4A and 4C are presented in Figs. 4B and 4D, respectively.

Figures 5A and 5C present data from  $\beta$ -gal assays to evaluate the effects of FK506 and the overexpression of CNB1 on the interactions of CN1c with CNA and CNA $\Delta$ C. Labeled schematic diagrams corresponding to the data shown in Figs. 5A and 5C are presented in Figs. 5B and 5D, respectively.

5        Figures 6A, 6C and 6E present data from  $\beta$ -gal assays to evaluate the effects of FK506, cyclosporin A (CsA), and the deletion of CNB1 on the interaction of CN1c with CNA and CNA $\Delta$ C. Labeled schematic diagrams corresponding to the data shown in Figs. 6A, 6C and 6E are presented in Figs. 6B, 6D and 6F, respectively.

10       Figures 7A, 7C, 7E and 7G present data from  $\beta$ -gal assays to evaluate the effects of FK506, cyclosporin A (CsA), rapamycin and the overexpression of CNB1 on the interaction of CN1c with CNA and CNA $\Delta$ C. Labeled schematic diagrams corresponding to the data shown in Figs. 7A, 7C, 7E and 7G are presented in Figs. 7B, 7D, 7F and 7H, respectively.

15       Figure 8A presents data from a  $\beta$ -gal assay to evaluate the effects of overexpression of full-length CNI on FK506-dependent interaction of FKBP with CNA. A labeled schematic diagram corresponding to the data shown in Fig. 8A is presented in Fig. 8B.

Figures 9A and 9C present data from  $\beta$ -gal assays to evaluate the effects of overexpression of full-length CNI on FK506-dependent interaction of FKBP with CNB1. Labeled schematic diagrams corresponding to the data shown in Figs. 9A and 9C are presented in Figs. 9B and 9D, respectively.

20       Figure 10 presents an image of a protein blot of CN1c and CNA co-immunoprecipitate probed with anti-CNA2 antibody.

Figure 11 presents an image of a yeast RNA blot hybridized with a CN1c probe.

Figure 12 presents the DNA sequence of a 3.5 kb fragment of yeast chromosome 11 containing the coding sequence for a yeast CNI protein.

25       Figure 13 presents the amino acid sequence of a yeast CNI protein.

#### **Brief Description of the Sequences**

SEQ ID NO:1 presents the nucleotide sequence of a *Sau3AI* fragment containing the coding sequence for CN1c.

30       SEQ ID NO:2 presents the amino acid sequence of CN1c encoded by SEQ ID NO:1.

SEQ ID NO:3 presents the coding sequence presented in SEQ ID NO:1.

SEQ ID NO:4 presents the nucleotide sequence of a gene encoding a complete CNI protein.

SEQ ID NO:5 presents the amino acid sequence encoded by SEQ ID NO:4.

SEQ ID NO:6 presents the coding sequence presented in SEQ ID NO:4.

SEQ ID NO:7 presents the nucleotide sequence of PCR primer CNI-PCR-A.

SEQ ID NO:8 presents the nucleotide sequence of PCR primer CNI-PCR-B.

5      SEQ ID NO:9 presents the nucleotide sequence of a gene encoding the yeast CNA1 subunit of calcineurin.

SEQ ID NO:10 presents the amino acid sequence encoded by SEQ ID NO:9.

SEQ ID NO:11 presents the nucleotide sequence of a gene encoding the yeast CNA2 subunit of calcineurin.

SEQ ID NO:12 presents the amino acid sequence encoded by SEQ ID NO:11.

10      SEQ ID NO:13 presents the nucleotide sequence of a gene encoding the yeast CNB1 subunit of calcineurin.

SEQ ID NO:14 presents an amino acid sequence encoded by SEQ ID NO:13.

SEQ ID NO:15 presents an amino acid sequence encoded by SEQ ID NO:13.

SEQ ID NO:16 presents the coding sequence presented in SEQ ID NO:13.

15      SEQ ID NO:17 presents the amino acid sequence encoded by SEQ ID NO:16.

SEQ ID NO:18 presents a nucleotide sequence encoding CNA1Δc.

SEQ ID NO:19 presents the amino acid sequence encoded by SEQ ID NO:18.

SEQ ID NO:20 presents a nucleotide sequence encoding CNA2Δc.

SEQ ID NO:21 presents the amino acid sequence encoded by SEQ ID NO:20.

20      SEQ ID NO:22 presents the nucleotide sequence of PCR primer G4-PCR-A.

SEQ ID NO:23 presents the nucleotide sequence of PCR primer G4-PCR-B.

### **Detailed Description of the Invention**

#### **I. DEFINITIONS**

25      A "calcineurin-targeted immunosuppressant" is a compound that possesses *in vivo* immunosuppressive activity, and that interacts with an immunophilin to form a complex which is capable of inhibiting calcineurin.

"Interacting proteins" are proteins capable of specifically binding to one another, or associating with one another, in a cell or *in vitro*.

30      A calcineurin interacting (CNI) protein or polypeptide is a protein or polypeptide that is effective to enhance immunosuppressive effects of a calcineurin-targeted immunosuppressant by potentiating an interaction of an immunophilin with calcineurin. Preferably, a CNI protein or polypeptide is a protein or polypeptide having an amino acid sequence that is homologous to the sequence presented herein as SEQ ID NO:5.

"Substantially isolated" is used in several contexts and typically refers to the at least partial purification of a CNI protein or polypeptide fragment away from unrelated or contaminating components (*e.g.*, cytoplasmic contaminants and heterologous proteins). Methods and procedures for the isolation or purification of compounds or components of interest are described below (*e.g.*, affinity purification of fusion proteins and recombinant production of CNI polypeptides).

In the context of the present invention, the phrase "nucleic acid sequences," when referring to sequences which encode a protein, polypeptide, or peptide, is meant to include degenerative nucleic acid sequences which encode homologous protein, polypeptide or peptide sequences as well as the disclosed sequence.

Two nucleic acid fragments are considered to have "homologous" sequences if they are capable of hybridizing to one another (i) under typical hybridization and wash conditions, as described, for example, in Sambrook, *et al.*, pages 320-328, and 382-389, or (ii) using reduced stringency wash conditions that allow at most about 25-30% basepair mismatches, for example:  $2 \times \text{SSC}$ , 0.1% SDS, room temperature twice, 30 minutes each; then  $2 \times \text{SSC}$ , 0.1% SDS, 37°C once, 30 minutes; then  $2 \times \text{SSC}$ , room temperature twice, 10 minutes each. Preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches. These degrees of homology can be selected by using wash conditions of appropriate stringency for identification of clones from gene libraries (or other sources of genetic material), as is well known in the art.

Two amino acid sequences or two nucleotide sequences (in an alternative definition for homology between two nucleotide sequences) are considered homologous (as this term is preferably used in this specification) if they have an alignment score of  $>5$  (in standard deviation units) using the program ALIGN, typically default mutation gap matrix and gap penalty (Dayhoff). The two sequences (or parts thereof) are more preferably homologous if their amino acids are greater than or equal to 40% using the ALIGN program mentioned above.

## II. OVERVIEW OF INVENTION

Experiments performed in support of the present invention demonstrate the identification and isolation of the nucleic acid sequence encoding a calcineurin interacting (CNI) protein. Further experiments performed in support of the present invention characterize the CNI protein, as well as a polypeptide containing only the c-terminal 306 amino acids of the CNI protein (CN1c). The experiments demonstrate that CN1c interacts specifically with the "A" subunits of calcineurin (CNA1 and CNA2), but not with calcineurin "B" subunit (CNB1).

The experiments also demonstrate that CN1c does not interact directly with FK506 binding protein (FKBP; Schreiber, *et al.*; with or without FK506), GAL4 binding domain (G4<sub>BD</sub>) or lamin C. The experiments also demonstrate that CN1c interacts with C-terminally truncated forms of CNA (CNA $\Delta$ C), which have lost their autoinhibitory domains, though the interaction is somewhat weaker than with full length CNA proteins.

Additional experiments show that the interaction between CN1c and CNA is enhanced when CNB1 is deleted, and diminished when CNB1 is overexpressed, that the interaction between CN1c and CNA or CNA $\Delta$ C is markedly enhanced by FK506 and by Cyclosporin A (CsA), but not rapamycin, and that overexpression of a full-length CNI protein enhances the interaction between CNA and FKBP (detectable only in the presence of FK506).

Additional experiments conducted in support of the present invention demonstrate that overexpression of the full-length CNI has no detectable effect on the interaction between CNB1 and CNA, and that in the presence of FK506 or CsA, overexpression of CNB1 no longer inhibits the interaction of CN1c with CNA.

It was also found that CNI deletion mutants are viable, both in wild-type and CN-deletion backgrounds, and that CNI deletion mutants in a CN-deletion background are more resistant to hygromycin B than normal CN-deletion mutants.

Co-immunoprecipitation experiments demonstrate that CN1c and CNA co-immunoprecipitate in the presence of FK506, and protein blot experiments show that CNI is expressed at low levels *in vivo*. RNA blot experiments show that CNI is encoded by a single message approximately 2.9 kb in length.

A comparison of the yeast CNI sequence with sequences present in nucleic acid and amino acid databases reveals no obvious homologous sequences have been identified in other organisms.

### III. CALCINEURIN

Experiments performed in support of the present invention were designed to identify polypeptides capable of interacting with calcineurin. Calcineurin (also called phosphoprotein phosphatase 2B or PP2B), has been characterized from many different tissues and organisms (Klee, *et al.*). It is a heterodimer of two subunits, of which the "A" subunit is about 61 kD in weight, possesses catalytic activity and also contains the association site for calmodulin. The "B" subunit contains four Ca<sup>2+</sup> binding sites and activates the A subunit. Calcineurin has little enzymatic activity, even in the presence of Ca<sup>2+</sup> and only becomes fully active when associated with calmodulin (Cyert).

Two A subunits (CNA1 and CNA2; Cyert, *et al.*, 1991) and one B subunit (CNB1, Cyert, *et al.*, 1992) have been cloned in yeast. Either CNA1 or CNA2 may associate with CNB1 to form a functional calcineurin heterodimer. Multiple isotypes of the A subunit have been cloned from a variety of organisms and are highly conserved (Klee, *et al.*). In particular, 5 calcineurin subunits have been cloned from human tissue (see reviews by, for example, Klee, *et al.*, and Guerini, *et al.*).

#### IV. IMMUNOSUPPRESSANT DRUGS

FK506, cyclosporin A (CsA) and rapamycin, derived from fungi, inhibit the activation 10 of T-cells by antigens. The compounds have proven highly effective at suppressing mammalian immune systems *in vivo*. In particular, CsA therapy in clinical settings has dramatically increased the success rate of transplantation therapy.

It is now known that FK506 and CsA exert their immunosuppressive effects, in part, by inhibiting the transcriptional activation of the interleukin-2 (IL-2) gene, whereas rapamycin 15 appears to function by inhibiting the response of T-cells to IL-2, presumably by inhibiting a transduction pathway mediated by the IL-2 receptor.

The molecular mechanism of FK506 and CsA immunosuppressive action involves a group of small, abundant intracellular proteins termed immunophilins, which bind with a high affinity to the immunosuppressants (Schreiber). At least two classes of immunophilins are 20 known to exist. One class, termed cyclophilins, binds to CsA, while another class, the FK506-binding proteins (FKBPs) binds FK506 and rapamycin. Many immunophilin genes, from a variety of organisms, have been cloned, and appear to be highly conserved from simple eukaryotes to mammals.

It is believed that FK506 and CsA-induced immunosuppression is due to the binding 25 of complexes, formed by binding of immunosuppressants FK506 and CsA bound to one of their respective immunophilins, to the catalytic subunit of calcineurin (Schreiber, *et al.*, Liu, *et al.*, Foor, *et al.*, Weiss, *et al.*). The binding of such a complex to an (A) subunit inhibits activation of calcineurin by increased intracellular calcium, which in turn prevents calcineurin from activating transcription factor NF-AT. Since IL-2 is one of the genes controlled by NF- 30 AT in T-cells, inhibition of the transcription factor inhibits the production of IL-2, resulting in immunosuppression (Clipstone, *et al.*).

FK506 and CsA are widely used in organ transplantation to prevent host rejection. However, both drugs are known to have many undesired side-effects such as neurotoxicity, nephrotoxicity, hypertension, and metabolic disorder. Accordingly formulations effective to

increase a target cell's sensitivity to these drugs may be useful in alleviating some of the aforementioned side-effects. Specifically, CNI and its homologues or derivatives, administered at appropriate levels, may be able to increase the sensitivity of CN to FK506/CsA and reduce the necessary dosage thus reducing or eliminating the side-effects of these drugs.

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#### V. TWO HYBRID PROTEIN INTERACTION ASSAYS

Two hybrid protein interaction assay methods (two hybrid protein-protein interaction screens) provide a simple and sensitive means to detect the interaction between two proteins in living cells. The assays are based on the finding that most eukaryotic transcription activators  
10 are modular (e.g. Brent, *et al.*), *i.e.*, that the activators typically contain activation domains that activate transcription, and DNA binding domains that localize the activator to the appropriate region of a DNA molecule.

The development of two hybrid protein interaction assays was made possible by the observation that the DNA binding domain does not need to be physically located on the same  
15 polypeptide as the activation domain (Ma, *et al.*, Triezenberg, *et al.*), raising the possibility that transcription of reporter genes could be used as an assay to detect protein interactions.

The utility of two hybrid systems for detecting interactions between two interacting proteins was fully realized by the observation that protein interactions could be detected if two potentially-interacting proteins were expressed as fusions, or chimeras (Fields, *et al.*). A first  
20 fusion protein contains one of a pair of interacting proteins fused to a DNA binding domain, and a second fusion protein contains the other of a pair of interacting proteins fused to a transcription activation domain. The two fusion proteins are independently expressed in the same cell, and interaction between the "interacting protein" portions of the fusions reconstitute the function of the transcription activation factor, which is detected by activation of  
25 transcription of a reporter gene.

At least two different cell-based two hybrid protein-protein interaction assay systems have been used to assess binding interactions and/or to identify interacting proteins. Both employ a pair of fusion hybrid proteins, where one of the pair contains a first of two "interacting" proteins fused to a transcription activation domain of a transcription activating  
30 factor, and the other of the pair contains a second of two "interacting" proteins fused to a DNA binding domain of a transcription activating factor.

The yeast GAL4 two hybrid system (Fields, *et al.*, Chien, *et al.*, Durfee, *et al.*, Bartel, *et al.*), utilized for experiments performed in support of the present invention, was developed

to detect protein-protein interaction based on the reconstitution of function of GAL4, a transcriptional activator from yeast, by activation of a *GAL1-lacZ* reporter gene.

Like several other transcription activating factors, GAL4 contains two distinct domains, a DNA binding domain and a transcription activation domain. Each domain can be independently expressed as a portion of a fusion protein composed of the domain, and a second, "bait" interacting protein. The two fusion proteins are then independently expressed together in a cell. When the two GAL4 domains are brought together by a binding interaction between the two "interacting" proteins, transcription of a reporter gene under the transcriptional control of GAL4 is initiated. The reporter gene typically has a promoter containing GAL4 protein binding sites (GAL upstream activating sequences, UAS<sub>G</sub>). Exemplary reporter genes are the *GAL1-lacZ*, and *GAL1-HIS3* reporter genes used in experiments described herein.

A second two hybrid system, described in detail in Ausubel, *et al.*, utilizes a native *E. coli* LexA repressor protein, which binds tightly to appropriate operators. A plasmid is used to express one of a pair of interacting proteins (the "bait" protein) as a fusion to LexA. The plasmid expressing the LexA-fused bait protein is used to transform a reporter strain of yeast, such as EGY48, that contains pSH18-34.

In this strain, binding sites for LexA are located upstream of two reporter genes. In the first reporter system, the upstream activation sequences of the chromosomal LEU2 gene—required in the biosynthetic pathway for leucine (Leu)—are replaced in EGY48 with *lexA* operators, permitting selection for viability when cells are plated on medium lacking Leu. In the second reporter system, EGY48 harbors a plasmid, pSH18-34, that contains a *lexA* operator-*lacZ* fusion gene, permitting discrimination based on color when the yeast is grown on medium containing Xgal (Ausubel, *et al.*).

To screen a library with the LexA system, the library uses the inducible yeast GAL1 promoter to express proteins as fusions to an acidic domain ("acid blob") that functions as a portable transcriptional activation motif ("act"), and to other useful moieties. Expression of library-encoded proteins is induced by plating transformants on medium containing galactose (Gal), so yeast cells containing library proteins that do not interact specifically with the bait protein fail to grow in the absence of Leu. Yeast cells containing library proteins that interact with the bait protein form colonies within 2 to 5 days, and the colonies turn blue when the cells are streaked on medium containing Xgal. The plasmids are isolated and characterized by a series of tests to confirm specificity of the interaction with the initial bait protein. Those found to be specific are ready for further analysis (e.g., sequencing).



LexA and GAL4 each have different properties that should be considered when selecting a system. LexA is derived from a heterologous organism, has no known effect on the growth of yeast, possesses no residual transcriptional activity, can be used in GAL4<sup>+</sup> yeast, and can be used with a Gal-inducible promoter. Because GAL4 is an important yeast transcriptional activator, experiments must be performed in *gal4* yeast strains to avoid background from endogenous GAL4 activating the reporter system.

Both two hybrid systems have been successfully used for isolating genes encoding proteins that bind a target protein and as simple protein binding assays (*e.g.*, Yang, *et al.*, Gyuris, *et al.*), and both can be applied to methods of the present invention.

Both gene isolation and protein binding assay applications of the GAL4 system are described in Examples below.

#### VI. SPECIFIC EMBODIMENTS

Example 1 demonstrates application of an exemplary two hybrid protein-protein interaction screen (Materials and Methods, section D) to the screening of three pGAD yeast fusion libraries, carrying fusions between the transcription activating domain of yeast protein GAL4 (G4AD) and yeast genomic DNA *Sau3AI* fragments, in all three reading frames. The libraries are screened to identify polypeptides, encoded by the *Sau3AI* fragments, capable of interacting with catalytic (A) subunits of calcineurin, expressed as fusions with the GAL4 protein binding domain (GBT-CNA fusions).

Three sets of yeast cells harboring pGBT-CNA1 *TRP1* (GBT-A1) hybrid plasmid and a GAL4-activated LacZ reporter gene are each transformed with one of the three reading-frame libraries. Construction of the plasmids used is described in Materials and Methods, sections B and C. Cells transformed with a plasmid encoding a protein fusion capable of interacting with the CNA subunit fusion are selected using a  $\beta$ -galactosidase ( $\beta$ -gal) assay on plates containing the chromogenic substrate X-gal (Materials and Methods, section E). Results of the  $\beta$ -gal assay are confirmed using a growth assay (Materials and Methods, section F). False positives are eliminated by colony purification (re-streaking for single colonies), PCR experiments using GAL4 primers, and testing against a number of test fusions by  $\beta$ -gal assays on transformed haploid or mated diploid reporter strains.

A yeast clone encoding a polypeptide capable of specifically interacting with CNA polypeptide fusions is identified and sequenced. The sequence of the *Sau3AI* fragment is presented as SEQ ID NO:1. The coding sequence forming the open reading frame is presented as SEQ ID NO:3. The polypeptide encoded by the open reading frame is presented as SEQ

ID NO:2. The open reading frame encodes 306 amino acids followed by a stop codon in frame with the coding sequence of GAL4, but does not contain an in-frame ATG start site upstream of the stop codon. This is consistent with the fragment encoding a c-terminal portion of a complete gene product. The clone is termed CN1c, with the lowercase "c" representing "c-terminal".

Figure 1A shows a schematic representation of the nucleic acid sequence encoding the GAL4AD-CN1c fusion protein. The stippled portion between GAL4AD and CN1c represents a linker discussed in the Materials and Methods section, as well as in Example 1. Also indicated in the Figure are the approximate locations of in-frame start (ATG) and stop codons, and *Sau3AI* restriction sites.

Example 1 further describes the identification of a  $\lambda$  clone encoding a full length sequence version of CN1c, termed CN1. The polypeptide encoded by the sequence is termed CN1 protein. The clone is identified by hybridization screening of a panel of  $\lambda$  clones spanning the yeast genome using a 1.22 kb  $^{32}\text{P}$ -labeled probe generated from CN1c.

Phage lysates of the  $\lambda$  clone are amplified, purified, restriction-mapped and used as a DNA source for subsequent cloning experiments. A 3.16 kb *MunI/EcoRV* fragment from the  $\lambda$  clone insert contains the coding sequence of CN1. The sequence of the 3.16 kb *MunI/EcoRV* fragment is encompassed by the 3.5 kb sequence presented as SEQ ID NO:4, and a schematic diagram of the sequence is shown in Figure 1B. This sequence contains the entire 2.75 kb coding sequence of CN1 (SEQ ID NO:6; graphically indicated in Figure 1B by the locations of the "ATG" and "Stop" codons).

A search of known DNA and protein sequences turns up no obvious matches or homologies to genes in other organisms. Accordingly, CN1 may represent a new type of calcineurin interacting protein.

The methods referred to above may also be applied to the screening of, for example, a human cDNA library using an appropriate two-hybrid protein interaction screen. The "bait" protein in the interaction screen (*e.g.*, the protein analogous to CNA1 in Example 1) may be of yeast origin (*e.g.*, CNA1), but is preferably of human origin (*e.g.*, a human calcineurin "A" subunit; Klee, *et al.*). The bait protein is expressed in the cell (*e.g.*, a yeast cell) used for the two hybrid interaction screen as a fusion to a domain of a transcription activating factor (*e.g.*, the DNA binding domain of GAL4). The library may be a human DNA library in a vector (*e.g.*, pGAD) effective to express library sequences as fusions to a complimentary domain of the transcription activating factor (*e.g.*, the activation domain of GAL4). Libraries of human sequences can be derived from a number of sources including genomic DNA, such as yeast

artificial chromosome (YAC) constructs carrying genomic human DNA, or cDNA generated from a variety of cell types (*e.g.*, activated T-cells).

Example 2 details a  $\beta$ -gal assay to determine the specificity of binding of CN1c to subunits of calcineurin. Exemplary results are shown in Figure 2A. The legend for Fig. 2A is shown in Figure 2B. Numbers in the legend refer to locations of yeast colonies expressing particular combinations of plasmid constructs (indicated in Example 2).

A comparison of the intensities of the blue  $\beta$ -gal reaction product indicates that CN1c interacts strongly with CNA1 (21, 22 and 23), and somewhat less strongly with CNA2 (24). Neither cells containing AS-lamin with GAD-CN1c (25), nor cells containing only GBT with GAD-CN1c (26) show a detectable signal above background. Two subunits of calcineurin (CNA1, CNB1) known to interact with each other are used as a positive control for the assay (20). The data presented in Figure 2A show that CN1c interacts specifically with CNA1 and CNA2, but not with G4BD or lamin C.

A similar set of experiments, illustrated in Fig. 3A, is conducted using constitutively-active CNA subunits, as well as calcineurin subunit CNB1. As described in the Materials and Methods section below, CNA1 $\Delta$ C and CNA2 $\Delta$ C are each missing a C-terminal portion of the protein containing an autoinhibitory domain. Exemplary results from these experiments are shown in Figure 3A. The legend for Fig. 3A is shown in Figure 3B. The data show that GBT-A1 $\Delta$ C and GAD-CN1c (28) gives a definite positive signal, while GBT-A2 $\Delta$ C and GAD-CN1c (29) is weaker, though still detectable above its background (*i.e.* GBT-A2 $\Delta$ C and GAD; 31). The signal from GBT-B1 and GAD-CN1c (32) is not detectable above vector background (GBT-B1 and GAD; 33). Positive controls GBT-A1 $\Delta$ C and GAD-B1 (34) and GBT-A2 $\Delta$ C and GAD-B1 (35) give strong signals. The data presented in Figure 3A show that CN1c interacts specifically with CNA1 $\Delta$ C and CNA2 $\Delta$ C, but not with CNB1.

Example 3 details the effects of immunosuppressant drugs on binding of CN1c to calcineurin in B1<sup>+</sup>, B1 Deletion and B1 Overproducing Yeast Strains. The yeast strains are assayed for  $\beta$ -gal activity as above to determine if the immunosuppressant drugs FK506, cyclosporin A (CsA) and rapamycin affect the binding of CN1c to subunits of calcineurin. The experiments are performed in yeast strains wild-type for the CNB1 subunit, null for the CNB1 subunit, and in yeast transformed with a high efficiency expression vector containing DNA encoding the CNB1 subunit.

Exemplary data are shown in Figures 4A, 4C, 5A and 5C. The legends for Figs. 4A and 4C are shown in Figures 4B and 4D, respectively. Figs. 4A and 5A illustrate experiment

performed without FK506, while experiments shown in Figs. 4C and 5C were performed with FK506.

The interactions of various combinations of proteins expressed by constructs indicated in Example 3 was studied in three yeast strains, one of which is null for the CNB1 subunit of calcineurin (Y153b; at 36-40), while the others (Y190; at 41-46 and Y526 at 47-51) are wild-type for CNA1, CNA2 and CNB1.

The data, shown in Fig. 4A (no added drugs), illustrate that deleting the endogenous host CNB1 gene potentiates, or enhances, interactions between CN1c and calcineurin subunits CNA1, CNA2, CNA1ΔC and CNA2ΔC. Comparison of corresponding colonies in Figs. 4A and 4C shows the effects of FK506 on CN1c-CNA/CNAΔC interactions. The drug enhances interactions under all except control conditions. The effect is most striking in yeast strains wild-type for the CNB1 subunit (e.g., compare 50a with 50b, and 51a with 51b).

The drug also markedly enhances, or potentiates CN1c-CNA/CNAΔC interactions under conditions where the CNB1 subunit is overexpressed. Figure 5A shows the effect of overexpressing CNB1 on CN1c-CNA/CNAΔC interactions in the absence of drug. Colonies expressing B1/YEp352 (53a, 55a-58a) have reduced signal as compared with controls expressing only YEp352 (52a, 54a). Inclusion of FK506 in the plating medium (Fig. 5C), however, enhances, or potentiates interactions in all colonies, except the negative controls (56).

The data presented in Figures 4A, 4C, 5A and 5C demonstrate that the interaction of CN1c with CNA and CNAΔC is markedly enhanced by FK506. The interaction is also enhanced by deletion of CNB1 and diminished by overexpression of CNB1, and the inhibitory effect of CNB1 overproduction is overcome by the stimulatory effect of FK506.

Stated another way, inclusion of a small molecule immunosuppressant (FK506) potentiates an interaction between two fusion hybrid proteins, where one of the two proteins contains an (A) subunit of calcineurin, and the other protein contains a CNI polypeptide. The potentiation is particularly strong when the cell is further modified to cause overexpression of a "B" subunit of calcineurin by said cell (e.g., the expresses B1/YEp352).

In the present case, a yeast cell is modified to cause overexpression of a "B" subunit of calcineurin (CNB1) by transforming the cell with B1/YEp352 (construction described below). A cell may be modified to cause overexpression of a "B" subunit of calcineurin in other ways as well, such as, for example, transformation with other types of expression vectors encoding a "B" subunit of calcineurin, or treatment with a substance that upregulates a promoter controlling expression of an endogenous (B) subunit of calcineurin.

In light of the effects of FK506 on CN1c-CNA/CNA $\Delta$ C interactions, two other immunosuppressants, cyclosporin A and rapamycin, were examined in similar experiments. The results of these experiments are shown in Figures 6A-6F and 7A-7H.

The data presented in Figures 6A and 6C are essentially equivalent to those presented in Figures 4A and 4C, respectively. Data shown in Figure 6E demonstrate that like FK506, the immunosuppressant cyclosporin A is also effective to enhance interaction of CN1c with CNA and CNA $\Delta$ C. Both FK506 and cyclosporin A are known to exert their immunosuppressive effects through calcineurin (Cyert).

Similarly, data shown in Figures 7A and 7C support data in Figures 5A and 5C, and further, demonstrate that there is no detectable interaction between FK506 binding protein (FKBP) and CN1c. Results shown in Fig. 7E demonstrate that cyclosporin A has a similar effect to FK506 in cells overexpressing CNB1 -- that is, it enhances the interactions between CN1c and CNA/CNA $\Delta$ C.

In contrast, data presented in Fig. 7G show that the immunosuppressant rapamycin has no detectable effect on CN1c-CNA/CNA $\Delta$ C interactions (compare Fig. 7G with Fig. 7A).

Taken together, the data presented in Figs. 6A, 6C, 6E 7A, 7C, 7E and 7G show that like FK506, cyclosporin A (CsA), but not rapamycin, enhances the interaction of CN1c with CNA and CNA $\Delta$ C, and that CN1c doesn't interact with FKBP with or without FK506.

Example 4 describes experiments to assess effects of CN1c on FKBP/FK506 binding to calcineurin. Figure 8A presents exemplary data from studies to assess the effect of CN1 on FKBP-mediated FK506 interactions with CNA2. The legend for Fig. 8A is shown in Figure 8B. Data in Figure 8A demonstrate that, in the absence of FK506, FKBP and CNA2 show no detectable interaction (84). In the presence of FK506, however, the proteins interact (85), presumably because FK506 forms a complex with FKBP, which then binds CNA2 (Cyert).

Data in Figure 8A further show that, in the absence of FK506, CN1 has no effect on the lack of interaction between FKBP and CNA2 (86). In the presence of FK506 (87), however, CN1 potentiates, or enhances the binding between FKBP and CNA2 (compare the intensity at 87 with that at 85). This effect suggests that CN1 and similar compounds may be employed to increase the sensitivity of calcineurin to immunosuppressant drugs that act on it, and in this way, decrease the amount of the immunosuppressant required for a particular level of immunosuppression.

Experiments illustrated in Figures 9A and 9B demonstrate that FK506 has little or no effect on the binding of CNA to CNB1. The legends for Fig. 9A and 9B are shown in Figures 9B and 9D, respectively. The data in Fig. 9A show that overexpression of the full-length CN1

clone markedly enhances the FK506-dependent interaction of FKBP with CNA, although it doesn't affect the interaction between CNA and CNB1.

Example 5 presents co-immunoprecipitation of CN1c (carrying an HA epitope tag) and CNA. Immunoprecipitation is carried out with anti-HA monoclonal antibody and the immune complex, resolved by SDS-PAGE, is detected with anti-CNA2 polyclonal antibody and visualized with goat anti-rabbit antibody using the "ECL" method (Amersham, Arlington Heights, IL). The results, shown in Figure 10, demonstrate that CN1c is capable of binding to CNA2 tightly enough for the complex to be co-immunoprecipitated. Similar methods may be employed to isolate a CNI analog from other cell sources, including mammalian (specifically human).

Example 6 describes yeast RNA blots hybridized with a CN1c probe. Exemplary data are shown in Figure 11. A single message of approximately 2.9 kb is detected. The data indicate that CNI is an expressed gene encoding a 2.9 kb message in yeast.

Example 8 details the construction of cni null mutants. The null mutants are employed to assess if *CNI* is required for viability in yeast, and to test hygromycin B sensitivity. The experiments indicate that CNI is not essential for viability, since CNI deletion mutant strains can survive, but that CNI deletions render a host more resistant to hygromycin B. The effect is particularly pronounced in both MCY300-1 (*cnal<sup>-</sup>cnb2<sup>-</sup>*) and DD12 (*cnb1<sup>-</sup>*), suggesting that CNI functions as a suppressor of CN mutant's sensitivity to hygromycin B.

It will be understood that all of the above methods and experimental manipulations are amenable to being done with interacting polypeptides from organisms other than yeast. In particular, calcineurin subunits, CNI polypeptides, immunophilins and the like may be of mammalian origin, *e.g.*, human origin.

## VII. UTILITY

Methods and compositions of the present invention may be applied in a number of different ways. Following the guidance presented herein, one of skill in the art may isolate nucleic acids encoding additional CNI polypeptides, for example, a human CNI polypeptide.

In one approach, a yeast strain carrying a mutation of the *CNI* gene, *e.g.*, a deletion, is used to clone heterologous sequences (*e.g.*, human sequences) by complementation. A library of genomic DNA or, preferably, cDNA from an organism (*e.g.*, human) and tissue (*e.g.*, lymphocyte cells) of choice is cloned into a vector that can be maintained in yeast. Preferably, the vector contains a yeast promoter effective to express the heterologous sequences in yeast cells. Several heterologous libraries suitable for expression in *Saccharomyces*

*cerevisiae* containing DNA from *S. pombe* (Beach, *et al.*) and *Drosophila* have been constructed.

The library is transformed into a suitable yeast strain carrying a *cni* mutation, and transformants are selected using a suitable complementation assay. For example, transformants  
5 may be screened for increased hygromycin sensitivity, as experiments described herein indicate that *cni* deletion mutants possess a decreased sensitivity to hygromycin B (Example 8). The screen may be made more effective by using a yeast strain that is hypersensitive to hygromycin B, such as a strain deficient for a subunit of calcineurin (Example 8).

Alternatively, human CNI DNA sequences may be isolated by directly screening a  
10 library, *e.g.*, a lymphocyte cDNA library, for clones hybridizing with a yeast CNI nucleic acid probe. The generation of an exemplary yeast CNI nucleic acid probe is described in Example 1.

In another approach, particularly advantageous for isolating sequences expressed at low levels, a CNI nucleic acid probe may be used to screen a genomic library, *e.g.*, a human  
15 genomic library, to isolate a sequence that may be used to design probes or primers that may match the target sequence better than the yeast sequence. Such primers may be used with, for example, PCR, to isolate longer fragments from a tissue-specific library.

In yet another approach, an antibody generated against CNI polypeptide is used to immunoprecipitate a CNI polypeptide from an organism and/or tissue of choice. The protein  
20 may then be micro-sequenced, and the sequence utilized to design degenerate primers useful for isolating a cDNA.

CNI polypeptides of the present invention, particularly CNI fragments that retain a desired binding activity, may be used as lead compounds useful for the development of small molecules having cellular functions similar to those of the CNI-polypeptides, that is, molecules  
25 effective to enhance immunosuppressive effects of calcineurin-targeted immunosuppressants by potentiating an interaction of an immunophilin with calcineurin.

CNI-polypeptides of the present invention may also be employed in a method of increasing sensitivity of cells to calcineurin-affecting immunosuppressant drugs. In this method, a CNI-polypeptide is introduced into the cell typically prior to or at the same time as  
30 contacting the cell with an immunosuppressant drug, such as FK506. The polypeptide may be delivered by any suitable means effective to deliver polypeptides to selected cells.

Alternatively, nucleic acids encoding CNI polypeptides may be used in appropriate expression vectors as a genetic therapy tool to potentiate the immunosuppressive effects of

calcineurin-targeting immunosuppressant drugs. The vectors may be targeted to selected cells, such as T-cells, to increase their sensitivity to a given systemic dose of an immunosuppressant.

Another utility of the present invention includes methods of screening for substances that up-regulate expression of CNI polypeptides, *i.e.*, substances that affect transcription. Such substances are useful for sensitizing cells to immunosuppressant drugs. In this method, the CNI promoter can be attached to a gene that functions as a selectable marker (for use in genetic selections to screen test substances) or to a reporter gene (for use in evaluating the effect on CNI transcription by test substances).

In another aspect of the present invention, the CNI-polypeptides, for example, mammalian homologue polypeptides of CNI, have potential use as therapeutic agents for both human and veterinary use. For example, CNI-polypeptides may be used in a method of enhancing immunosuppression in a test subject. In this method, the CNI-polypeptide is administered to the subject in a pharmaceutically-acceptable formulation and at a concentration effective to potentiate the interaction of an immunosuppressant/immunophilin complex with a subunit of calcineurin. The method may also include contacting the CNI-polypeptide with a cell under conditions effective to permit uptake of the protein into the cell in order to increase sensitivity of the cell to immunosuppressants. A CNI polypeptide used in such methods may be modified to be more suitable for administration or to be more effective in a cell. For example, a CNI polypeptide may be modified to eliminate PEST motifs, which are typically found in proteins with short half-lives, to extend the effective lifetime of the polypeptide in the target cell.

The following examples illustrate, but in no way are intended to limit the present invention.

#### MATERIALS AND METHODS

Unless indicated otherwise, chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, MO) or Mallinckrodt Specialty Chemicals (Chesterfield, MO), restriction endonucleases were obtained from New England Biolabs (Beverly, MA), and other modifying enzymes and biochemicals were obtained from Pharmacia Biotech (Piscataway, NJ) or Boehringer Mannheim (Indianapolis, IN). FK506 was obtained from Fujisawa USA, Inc. (Deerfield, IL), cyclosporin A was obtained from Sandoz (Basel, Switzerland), and rapamycin was obtained from Wyeth-Ayerst (Princeton, NJ). Materials for media for yeast growth and culture were obtained from DIFCO (Detroit, MI). Unless otherwise indicated, manipulations



of yeast, bacteria, nucleic acids, proteins and antibodies were performed using standard methods and protocols (*e.g.*, Guthrie, *et al.*, Sambrook, *et al.*, Ausubel, *et al.*, Harlow, *et al.*, and Rose, *et al.*).

#### 5           A.     Buffers

Z buffer: 60 mM Na<sub>2</sub>HPO<sub>4</sub>-7H<sub>2</sub>O, 40 mM NaH<sub>2</sub>PO<sub>4</sub>-H<sub>2</sub>O, 10 mM KCl, 1 mM MgSO<sub>4</sub>-7H<sub>2</sub>O and 50 mM β-mercaptoethanol (pH 7.0).

#### B.     Plasmids, Libraries and Yeast Strains

10           Plasmids pGBT9 (GBT), carrying GAL4 DNA-binding domain (amino acid residues 1-147; G4BD) and TRP1, and pGAD (GAD), carrying GAL4 activation domain (amino acid residues 768-881; G4AD) and LEU2; three pGAD libraries carrying fusions between G4AD and yeast genomic *Sau3AI* partial-digest fragments in each frame; and the yeast *GAL1-lacZ* reporter strain SFY526 (Y526; *MATa ura3-52 ade2-101 lys2-801 his3-200 trp1-901 leu2-3,112*  
15 *can1 gal4-542 gal80-538 URA3::GAL1-lacZ*) were obtained from Stanley Fields (State University of New York at Stony Brook, Stony Brook, NY; Chien, *et al.*, Bartel, *et al.*). The libraries were constructed with linkers between the GAL4 activation domain and the *Sau3AI* fragments. The sequences of the linkers were 5'-ATCG-3' for the first library, 5'-ATCCG-3' for the second library, and 5'-ATCCCG-3' for the third library. In this way, the yeast  
20 genomic *Sau3AI* fragments were cloned in all three reading frames relative to G4AD.

Plasmids pAS2 (AS) carrying G4BD and TRP1, and pAS-lamin (AS-lamin) containing a sequence encoding a G4BD-lamin C fusion; and yeast reporter strains Y190 (*MATa ura3-52 ade2-101 his3-Δ200 trp1-901 leu2-3,112 cyh2Δ, gal4Δ gal80Δ URA3::GAL-lacZ LYS2::GAL-HIS3*), a derivative of Y153 carrying dual indicator genes (GAL-lacZ and GAL-  
25 HIS3), and Y187 (*MATα ura3-52 ade2-101 lys2-801 his3-200 trp1-901 leu2-3,112 gal4Δ gal80Δ URA3::GAL-lacZ*) carrying *GAL-lacZ* reporter were obtained from Stephen Elledge (Baylor College of Medicine, Houston, TX; Durfee, *et al.*). Yeast strain Y153b1 (*cnb1::ADE2*) was derived from Y153.

*E coli* strain JBe181 (leuB600 trpC9830) was obtained from Ira Herskowitz (University  
30 of California at San Francisco, San Francisco, CA). Protease-deficient yeast strain BJ2407 (Guthrie, *et al.*) was obtained from the Yeast Genetic Center (University of California at Berkeley, Berkeley, CA).

### C. GAL4-Calcineurin Fusions

GAL4-calcineurin (GAL4-CN) fusions, GBT-A1 (G4BD-CNA1), GBT-A2 (G4BD-CNA2), GBT-B1 (G4BD-CNB1), GAD-A1 (G4AD-CNA1), GAD-A2 (G4AD-CNA2), and GAD-B1 (G4AD-CNB1) were constructed as follows. Plasmids containing inserts encoding  
 5 CN subunits CNA1 (SEQ ID NO:9; Cyert, *et al.*, 1991), CNA2 (SEQ ID NO:11; Cyert, *et al.*, 1991) and CNB1 (SEQ ID NO:13; Cyert, *et al.*, 1992) were subjected to site-directed mutagenesis (Kunkle) to introduce a *Bam*HI site just upstream of each subunit's initiation codon in the second reading frame. DNA prepared from the mutated plasmids was digested with *Bam*HI and *Xho*I, and the resulting *Bam*HI-*Xho*I fragments, each containing a full-length coding  
 10 sequence, were cloned into GBT or GAD that had been cut with *Bam*HI and *Sal* I. The resulting plasmids encoded in-frame fusions of the CN subunits with G4BD or G4AD.

Plasmids encoding CNA protein variants with truncated C-termini (GBT-A1ΔC, GBT-A2ΔC) were constructed by introducing stop codons after amino acid residues 509 (CNA1) and 502 (CNA2). The 44-residue deletion in GBT-A1ΔC removed the autoinhibitory domain of  
 15 CNA1, while the 102-residue deletion in GBT-A2ΔC removed both the autoinhibitory and the calmodulin-binding domains of CNA2 (Cyert, *et al.*, 1991).

Plasmid GBT-FKBP, containing an FK506 binding protein (FKBP) gene fused to the GAL4 binding domain, was constructed by introducing a *Bgl*II site upstream of the initiation codon and a *Bam*HI site downstream of the stop codon of FKBP12 (Heitman, *et al.*) and  
 20 ligating the *Bgl*II-*Bam*HI fragment into GBT cut with *Bam*HI.

Plasmid B1/YEp352 was constructed to contain the full coding sequence of CNB1 (SEQ ID NO:13, Cyert, *et al.*, 1992) as a 1.4 kb *Bam*HI-*Eco*RI fragment encompassing the sequence presented as SEQ ID NO:13 (812 bp; contains the coding sequence), in the multicopy plasmid YEp352(HIS), which is derived from YEp352 (URA) (Hill, *et al.*).

25 Plasmid CNI/YEp352(HIS) (also referred to as CNIH) was constructed by ligating a 3.16 kb *Mun*I-*Eco*RV fragment, containing the full coding sequence of CNI, from plasmid CNI7.1 (construction described below) into YEp352(HIS) cut with *Eco*RI and *Sma*I. Plasmids CNI/YEp352(TRP) (also referred to as CNIT) and CNI/YEp352(URA) (also referred to as CNIU) were similarly constructed using the 3.16 kb fragment and YEp352(TRP) or  
 30 YEp352(URA), respectively (Hill, *et al.*).

Plasmids A1/YEp351 and A2/YEp352 were constructed to contain the full coding sequences of CNA1 (SEQ ID NO:9, Cyert, *et al.*, 1991) in YEp351 (Hill, *et al.*) and CNA2 (SEQ ID NO:11, Cyert, *et al.*, 1991) in YEp351 and YEp352, respectively. A1/YEp351 was constructed by ligating a 2.9 kb *Sac*I-*Hind*III fragment from clone CNA1 (Cyert, *et al.*, 1991)

into YEp351(HIS) cut with *SacI* and *HindIII*. A2/YEp352 was similarly constructed by ligating a 3 kb *SpeI-HindIII* fragment from clone CNA2 (Cyert, *et al.*, 1991) into YEp352(HIS) cut with *XbaI* and *HindIII*.

All GAL4-CN fusions were verified by DNA sequencing (Sanger, *et al.*) using  
5 "SEQUENASE 2.0" sequencing kits (United States Biochemical, Cleveland, OH), and were subjected to the following tests. The functionality of the fusion proteins was assayed by determining whether they could complement the appropriate *cn*<sup>-</sup> mutant phenotypes, using assays to measure the sensitivity to pheromone and Mn<sup>2+</sup> (Reneke, *et al.*, Cyert, *et al.*, 1991). All of the GAL4-CN fusions were functional in this assay.

10 The fusion proteins were also tested for their ability to activate the reporter gene in the absence of the complementary GAL-4 domain fusion (*i.e.*, in the presence of the complementary GAL4 domain not fused to a second protein, for example, G4BD-A1 vs G4AD) using the two-hybrid interaction assay described below. Only GBT-B1 and GAD-A1 were able to activate the reporter gene at low levels without the complimentary GAL-4 domain fusion --  
15 assays with the other fusion proteins in the absence of the complimentary GAL-4 domain fusion showed no detectable levels of expression.

The two-hybrid interaction assay was also used to test the ability of the fusions to interact specifically with another fusion containing complimentary GAL4 and CN domains (*e.g.*, G4BD-A1 interacting with G4AD-B1). All CN hybrids were able to react specifically  
20 and result in an activation of the reporter gene that was clearly detectable above background. The high specificity witnessed in these experiments indicates that the GAL4 two-hybrid system can reliably be used to assay interactions between CN and other proteins.

#### D. Yeast GAL4 Two-Hybrid System for Detecting Protein-Protein Interaction

25 In the library screen, described in more detail in Example 1A, the yeast strain Y190, harboring the hybrid plasmid carrying the GAL4 binding domain fused to the A1 subunit of calcineurin (G4BD-CNA1), was transformed with fusion libraries carrying yeast genomic DNA *Sau3AI* fragments fused to the GAL4 activation domain. Transformants that were able to express the reporter genes, *i.e.*, able to grow on -His + 3-AT and to score blue in  $\beta$ -gal assay,  
30 were selected as candidate positives. These candidate positives potentially contain library DNA fragments encoding proteins that physically interact with CNA1.

In another application described herein, the two-hybrid system was used to test for interactions between CNA (fused to one of the GAL4 domains) and CNB1 (fused to the other GAL4 domain), and between CNA and FKBP. Additional experiments tested a clone, CN1c,

isolated using the library screen, against a series of proteins fused to the complementary GAL4 domain under various conditions to test whether CN1c interacts with CNA subunits, and if so, how the interactions are affected by various conditions.

5           E.     Color Development ( $\beta$ -gal) Assay

Yeast reporters harboring both G4BD and G4AD fusions (and a third non-fusion plasmid in some cases) were monitored for  $\beta$ -gal activity as follows. Purified yeast transformants were patched onto selective plates with or without other test reagents. After growing 3 days at 30°C, colonies were lifted onto nitrocellulose filters, permeabilized in liquid  
10   nitrogen as above, placed on Whatman No. 1 paper in petri dishes containing 0.1% X-Gal in Z buffer (see above), and incubated at 30°C for 12 hours. Blue color begins to appear in positive colonies between about one half and ten hours into the incubation period.

Exemplary images obtained using the color development assay are presented in Figures 2A, 3A, 4A, 4C, 5A, 5C, 6A, 6C, 6F, 7A, 7C, 7E, 7G, 8A, 9A and 9C.

15

F.     Growth Assay

A growth assay, applicable to yeast strains Y190 and Y153b1 which carry both *GAL-HIS3* and *GAL-lacZ* reporters, was sometimes used as a complement to the color assay described above. Yeast transformants were streaked onto selective plates containing 40-50 mM  
20   3-AT and no Histidine, and incubated at 30°C for 3-7 days. Growth (corresponding to the level of *HIS3* expression) was monitored as an indicator of the interaction between fusion proteins. In cases where both assays were used, the amount of cell growth typically correlated well with the color intensity in the  $\beta$ -gal assay.

25           G.     Yeast Growth, Drug Treatment

Yeast were typically grown in YPD (rich non-selective) or synthetic complete (SC) medium with selected component drop-outs, depending on the plasmid introduced, following standard procedures (Sherman, *et al.*, Ausubel, *et al.*).

Experiments utilizing treatment with drugs or additives were performed by including  
30   the drug or additive in the medium. For plating, the agar was autoclaved, allowed to cool to 50°C, and the drug or additive was added before pouring the plates. Unless otherwise indicated, drugs and additives were added to result in the following final medium concentrations: FK506: 1  $\mu$ g/ml, cyclosporin A: 10  $\mu$ g/ml, rapamycin: 10 ng/ml, and hygromycin B: 40  $\mu$ g/ml.

## H. Antibodies

Polyclonal and monoclonal antibodies, for use in the present invention, can be prepared by standard methods (Harlow, *et al.*) utilizing the CNI polypeptides of the present invention, for example, a substantially purified CNI/ $\beta$ -galactosidase fusion protein (Example 9).  
5 Antibodies can also be generated by recombinant techniques (Cabilly, *et al.*; Better, *et al.*; Skerra, *et al.*). In addition to whole antibody molecules, antibody fragments retaining the immunological specificity of the whole antibody may also be used in the practice of the present invention (*e.g.*, Fab and F(ab')<sub>2</sub> fragments of IgG (Pierce Chemical, Rockford, IL)). The antibodies can be purified by standard methods to provide antibody preparations which are  
10 substantially free of serum proteins that may affect reactivity (*e.g.*, affinity purification (Harlow *et al.*)).

### EXAMPLE 1

#### Isolation of CN1c

##### 15 A. Library Screening

Yeast strain Y190 was transformed with pGBT-CNA1 *TRP1* (GBT-A1) hybrid plasmid using the transformation protocol described by Schiestl, *et al.* Transformants were selected, colony purified, and a single transformant was selected to make (Y190 GBT-A1)-competent cells, following the procedure described in Guthrie, *et al.*.

20 The three pGAD yeast fusion libraries described above, carrying fusions between G4AD and yeast genomic DNA *Sau3A*I fragments in each reading frame, were then used to transform (Schiestl, *et al.*) the Y190 GBT-A1-containing cells. Transformants were plated onto SC-Trp-Leu-His plates containing 40 mM 3-aminotriazole (3-AT; Sigma Chemical Co., St. Louis, MO) and incubated at 30°C for 6 days to screen for HIS<sup>+</sup> colonies (Durfee, *et al.*).

25 His<sup>+</sup> colonies were replica plated onto nitrocellulose filters (Schleicher & Schuell, Keene, NH), frozen in liquid nitrogen for approximately 30 seconds, and incubated at 30°C for 12 hours with Z buffer (see above) containing the chromogenic substrate X-Gal (0.1%) to assay  $\beta$ -gal activity (Breedon, *et al.*).

Candidate positive (blue) colonies were re-streaked for single colonies. Single colonies  
30 were purified and retested using the above protocol. Colonies which reproducibly tested positive were screened using PCR with primers directed against the internal portion of GAL-4 (*i.e.* the portion between the DNA binding domain and the activation domain). The sequences of the primers, G4-PCR-A and G4-PCR-B, are given as SEQ ID NO:22 and SEQ ID NO:23,

respectively. Colonies yielding a PCR product were identified as containing intact GAL4, and were eliminated.

The GBT-A1 *TRP*<sup>+</sup> plasmid was eliminated by growing in *Trp*<sup>+</sup> liquid media for 2-3 days, plating on -Leu media and then replica-plating on -Leu and -Trp plates to identify and eliminate colonies that had lost the GBT-A1 plasmid, yet still gave a positive signal.

Plasmid DNA was extracted from the remaining *Leu*<sup>+</sup> candidates. The plasmid DNA was transformed into *E coli* JBe181 and plated on -Leu media to select for library plasmids. The library plasmids isolated by this method were introduced back to the yeast reporter strains either alone or with test G4BD fusions: GBT, GBT-A1, and AS-lamin.

A parallel specificity assay was conducted by mating. Candidate strains, as described above, were 3-AT growth positive and X-gal positive when both the library and GBT-A1 plasmids were present. After elimination of the GBT-A1 plasmid from these strains, strains that were *Leu*<sup>+</sup> *Trp*<sup>-</sup> 3-AT growth<sup>-</sup> and  $\beta$ -gal<sup>-</sup> were mated to the following strains: Y187 (*MAT $\alpha$* ) carrying GBT, GBT-A1, or AS-lamin, and the diploids were assayed.

Among the 3-AT positive,  $\beta$ -gal positive candidates identified by the secondary screening method just described, one clone (III-21S, later termed GAD-CN1c) was specifically positive in conjunction with GBT-A1 in both the transformation assay and the mating assay.

#### B. Sequence of CN1c

Clone III-21S was sequenced as above. The sequence is presented herein as SEQ ID NO:1, and a schematic representation of the clone is shown in Figure 1A. The *Sau3AI* library insert encodes 306 amino acids followed by a stop codon in frame with the coding sequence of GAL4, but does not contain an in-frame ATG start site upstream of the stop codon. This is consistent with the fragment encoding a c-terminal portion of a complete gene product. Accordingly, the clone was termed CN1c, with the lowercase "c" representing "c-terminal".

The stippled portion between GAL-4AD and CN1c in Figure 1A represents the linker discussed in Materials and Methods, above. Also indicated in the Figure are the approximate locations of in-frame start (ATG) and stop codons, and *Sau3AI* restriction sites.

#### C. Isolation of a Full Length Clone

A <sup>32</sup>P-labeled CN1c probe was generated from the 1.22 kb CN1c insert of clone III-21S by polymerase chain reaction (PCR) using primers represented as SEQ ID NO:7 and SEQ ID NO:8. The probe was used to map the gene to the right arm of chromosome 11 by hybridization screening (Sambrook, *et al.*) a panel of  $\lambda$  clones (American Type Culture

Collection (ATCC), Rockville, MD) spanning the entire yeast genome. Two clones, 70500 and 70590, gave positive hybridization signals. A phage lysate of clone 70500 in  $\lambda$  MG3 was obtained from the ATCC, was amplified, purified, restriction-mapped and used as a DNA source for subsequent cloning experiments.

5       The phage DNA was digested with *SacI*, yielding a 7.1 kb fragment containing the entire CNI gene. This fragment was cloned into "BLUESCRIPT SK-" (Stratagene, La Jolla, CA) cut with *SacI*, yielding plasmid CNI7.1. Plasmid CNI7.1 was digested with *MunI* and *EcoRV*, releasing a 3.16 kb fragment containing the entire coding sequence of CNI. The 3.16 kb fragment was then cloned into each of YEp352(HIS), YEp352(TRP), YEp352(URA), and  
10 "BLUESCRIPT SK-", each cut with *EcoRI* and *SmaI*, yielding plasmids CNIH, CNIT, CNIU and CNI3.2, respectively. The sequence of the 3.16 kb *MunI/EcoRV* fragment is encompassed by the 3.5 kb sequence presented as SEQ ID NO:4. The *MunI* site is at nucleotide 100 of SEQ ID NO:4, and the *EcoRV* site is at nucleotide 3263 of SEQ ID NO:4.

A schematic diagram of the sequence presented as SEQ ID NO:4 is shown in Figure  
15 1B. This sequence contains the 3.16 kb *MunI/EcoRV* fragment used in many of the experiments described herein (depicted in Figure 1B as the portion between the *MunI* and *EcoRV* sites), which contains the entire 2.75 kb coding sequence of CNI (SEQ ID NO:6; graphically indicated in Figure 1B by the locations of the "ATG" and "Stop" codons).

Figure 13 also shows the location of certain features of the sequence. For example,  
20 "PEST" motifs (Rogers, *et al.*, Dice) are indicated by bars over the corresponding sequence.

A search of known DNA and protein sequences turned up no obvious matches or homologies to genes in other organisms. Accordingly, CNI may represent a new type of calcineurin-binding protein.

25

## EXAMPLE 2

### Binding of CNIC to Calcineurin

Y190 yeast carrying the plasmids indicated below were assayed for  $\beta$ -gal activity by color development assay described above to determine the specificity of binding of CNIC to subunits of calcineurin.

30       Exemplary data, in the form of images of filters having yeast colony replicas that had undergone the  $\beta$ -gal color development assay are shown in Figure 2A. The legend for Fig. 2A is shown in Figure 2B. Numbers in the legend refer to locations of yeast colonies expressing particular combinations of plasmid constructs. The constructs are as follows: 20:GBT-A1 and

GAD-B1, 21:GBT-A1 and GAD-CN1c, 22:GBT-A1 and GAD-CN1c, 23:GBT-A1 and GAD-CN1c 24:GBT-A2 and GAD-CN1c 25:AS-lamin and GAD-CN1c 26:GBT and GAD-CN1c.

Yeast colonies used in the assay were derived by several different methods. Those at location 22 were purified colonies from the original library screen, those at 21 were colonies  
5 transformed with mini-prep DNA of the isolated GAD-CN1c plasmid, and the remaining colonies (23, 24, 25 and 26) were transformed with maxi-prep (Qiagen, Chatsworth, CA) DNA of GAD-CN1c.

A comparison of the intensities of the blue  $\beta$ -gal reaction product indicates that CN1c interacted strongly with CNA1 regardless of the source of the CN1c plasmid DNA (20, 21, 22  
10 and 23), and somewhat less strongly with CNA2 (24). Neither cells containing AS-lamin with GAD-CN1c (25), nor cells containing only GBT with GAD-CN1c (26) showed a detectable signal above background. Two subunits of calcineurin (CNA1, CNB1) known to interact were used as a positive control for the assay (20).

In summary, the data above show that CN1c interacted specifically with CNA1 and  
15 CNA2, but not with G4BD or lamin C.

A similar set of experiments was conducted using constitutively-active CNA subunits, as well as calcineurin subunit CNB1. As described in Materials and Methods, above, CNA1 $\Delta$ C and CNA2 $\Delta$ C were each missing a C-terminal portion of the protein containing an autoinhibitory domain. Exemplary results from these experiments are shown in Figure 3A.  
20 The legend for Fig. 3A is shown in Figure 3B. Locations of yeast colonies expressing specific constructs are as follows: 27:GBT and GAD-CN1c, 28:GBT-A1 $\Delta$ C and GAD-CN1c, 29:GBT-A2 $\Delta$ C and GAD-CN1c, 30:GBT-A1 $\Delta$ C and GAD, 31:GBT-A2 $\Delta$ C and GAD, 32:GBT-B1 and GAD-CN1c, 33:GBT-B1 and GAD, 34:GBT-A1 $\Delta$ C and GAD-B1, and 35:GBT-A2 $\Delta$ C and GAD-B1.

25 The data show that GBT-A1 $\Delta$ C and GAD-CN1c (28) gave a definite positive signal, while GBT-A2 $\Delta$ C and GAD-CN1c (29) was weaker, though still detectable above its background (*i.e.* GBT-A2 $\Delta$ C and GAD; 31). The signal from GBT-B1 and GAD-CN1c (32) was not detectable above vector background (GBT-B1 and GAD; 33). Positive controls GBT-A1 $\Delta$ C and GAD-B1 (34) and GBT-A2 $\Delta$ C and GAD-B1 (35) gave strong signals.

30 These data show that CN1c interacted specifically with CNA1 $\Delta$ C and CNA2 $\Delta$ C, but not with CNB1.



**EXAMPLE 3****Effects of Immunosuppressant Drugs on Binding of CN1c to Calcineurin in B1<sup>Δ</sup>, B1 Deletion and B1 Overproducing Yeast Strains**

Three yeast strains carrying the plasmids indicated below were assayed for  $\beta$ -gal activity as above to determine if the immunosuppressant drugs FK506, cyclosporin A (CsA) and rapamycin affect the binding of CN1c to subunits of calcineurin. The experiments were performed in yeast strains wild-type for the CNB1 subunit, null for the CNB1 subunit, and in yeast transformed with a high efficiency expression vector containing DNA encoding the CNB1 subunit.

Exemplary data, in the form of filter images produced as above are shown in Figures 4A, 4C, 5A and 5C. Plates used to make the filters shown in Figs. 4A and 4C were replicas from one master plate, while plates used to make the filters shown in Figs. 5A and 5C were replicas from another plate. The plates used to generate filters shown in Figs. 4A and 5A were without FK506, while the plates used to generate filters shown in Figs. 4C and 5C contained 1  $\mu$ g/ml FK506.

The legends for Figs. 4A and 4C are shown in Figures 4B and 4D, respectively. Since the imaged filters in Figs. 4A and 4C were from replica plates, corresponding locations on each filter contain material from the same yeast colonies. Accordingly, the locations are referred to by the same "base" numbers in the legends. To facilitate reference to a specific location on a specific filter, the base numbers are followed by a lowercase letter that is different for each of the individual filters. For example, in the present figure, "a" follows the base numbers to identify locations on the filter shown in Fig. 4A, while a "b" follows the base numbers to identify locations on the plate in Fig. 4C. This labeling scheme is used in other experiments detailed herein where multiple filter lifts are shown.

The interactions of various combinations of proteins expressed by constructs indicated below was studied in three yeast strains. Strain Y153b<sup>+</sup>, at 36-40, is null for the CNB1 subunit of calcineurin. Strains Y190 (41-46) and Y526 (47-51) are wild-type for CNA1, CNA2 and CNB1.

Hybrid proteins expressed by colonies at specific locations are as follows: 36:GBT-A1 and GAD-CN1c, 37:GBT-A2 and GAD-CN1c, 38:GBT-A1 $\Delta$ C and GAD-CN1c, 39:GBT and GAD-CN1c, 40:GBT-A2 $\Delta$ C and GAD-CN1c, 41:GBT-A1 and GAD-CN1c, 42:GBT and GAD-CN1c, 43:GBT-A2 and GAD-CN1c, 44:GBT-A1 $\Delta$ C and GAD-CN1c, 45:GBT-A1 and GAD-B1, 46:GBT-A2 $\Delta$ C and GAD-CN1c, 47:GBT-A1 and GAD-CN1c, 48:GBT and GAD-CN1c,

49:GBT-A2 and GAD-CN1c, 50:GBT-A1ΔC and GAD-CN1c, and 51:GBT-A2ΔC and GAD-CN1c.

Yeast strain Y526 was used for all experiments shown in Figs. 5A, 5B, 5C and 5D. The expression vector B1/YEp352(HIS) was not used in strains Y190 or Y153b1 because they  
5 are HIS<sup>+</sup> in the absence of 3-AT.

The base numbers in Figures 5B and 5D correspond to locations of colonies expressing the following constructs: 52:GBT-A1, GAD-CN1c and YEp352, 53:GBT-A1, GAD-CN1c and B1/YEp352, 54:GBT-A2, GAD-CN1c and YEp352, 55:GBT-A2, GAD-CN1c and B1/YEp352, 56:GBT, GAD-CN1c and B1/YEp352, 57:GBT-A1ΔC, GAD-CN1c and B1/YEp352, and  
10 58:GBT-A2ΔC, GAD-CN1c and B1/YEp352.

A comparison of data shown in Fig. 4A (no added drugs) shows the effect of deleting the endogenous host CNB1 gene on interactions between CN1c and calcineurin subunits CNA1, CNA2, CNA1ΔC and CNA2ΔC. Note that interactions in panels 36a-40a (CNB1 null strain) were all stronger (with the exception of the negative control in 39) than interactions in  
15 corresponding panels 41a-51a (strains wild-type for CNB1). This result indicates that interaction between CN1c and CNA subunits were enhanced by the deletion of the CNB1 subunit.

Comparison of corresponding panels in Figs. 4A and 4C shows the effects of FK506 on CN1c-CNA/CNAΔC interactions. The drug enhanced interactions under all except control  
20 (39, 42 and 48) conditions. The effect was most striking in yeast strains wild-type for the CNB1 subunit (*e.g.*, compare 50a with 50b, and 51a with 51b).

The drug also markedly enhanced CN1c-CNA/CNAΔC interactions under conditions where the CNB1 subunit was overexpressed. Figure 5A shows the effect of overexpressing CNB1 on CN1c-CNA/CNAΔC interactions in the absence of drug. Colonies expressing  
25 B1/YEp352 (53a, 55a-58a) had reduced signal as compared with controls expressing only YEp352 (52a, 54a). Inclusion of FK506 in the plating medium (Fig. 5C), however, enhanced interactions in all colonies, except the negative controls (56).

Taken together, the above data demonstrate that the interaction of CN1c with CNA and CNAΔC was markedly enhanced by FK506. The interaction was also enhanced by deletion  
30 of CNB1 and diminished by overexpression of CNB1, and the inhibitory effect of CNB1 overproduction was overcome by the stimulatory effect of FK506.

In light of the effects of FK506 on CN1c-CNA/CNAΔC interactions, two other immunosuppressants, cyclosporin A and rapamycin, were examined in similar experiments. The results of these experiments are shown in Figures 6A-6F and 7A-7H. Filters shown in

Figs. 6A-6F were from replica plates, as were those in Figs. 7A-7H. Colonies shown in Figs. 6A and 7A were plated without drugs; those in Figs. 6C and 7C were plated with FK506 (1  $\mu$ g/ml), those in Figs. 6E and 7E with CsA (10  $\mu$ g/ml), and those in Fig. 7G with rapamycin (10 ng/ml). Yeast strains used were as follows: In Figs. 6A-6F, panels 59-63 were Y153b1, 5 64-69 were Y190, and 70-74 were Y526. In Figs. 7A-7H, panels 77-83 were Y526, and panels 75 and 76 were Y190.

The base numbers in Figures 6B, 6D and 6F correspond to locations of colonies expressing the following constructs: 59:GBT-A1 and GAD-CN1c, 60:GBT-A2 and GAD-CN1c, 61:GBT-A1 $\Delta$ C and GAD-CN1c, 62:GBT and GAD-CN1c, 63:GBT-A2 $\Delta$ C and GAD-CN1c, 10 64:GBT-A1 and GAD-CN1c, 65:GBT and GAD-CN1c, 66:GBT-A2 and GAD-CN1c, 67:GBT-A1 $\Delta$ C and GAD-CN1c, 68:GBT-A1 and GAD-B1, 69:GBT-A2 $\Delta$ C and GAD-CN1c, 70:GBT-A1 and GAD-CN1c, 71:GBT and GAD-CN1c, 72:GBT-A2 and GAD-CN1c, 73:GBT-A1 $\Delta$ C and GAD-CN1c, and 74:GBT-A2 $\Delta$ C and GAD-CN1c.

The base numbers in Figures 7B, 7D, 7F and 7H correspond to locations of colonies 15 expressing the following constructs: 75:GBT-FKBP and GAD, 76:GBT-FKBP and GAD-CN1c, 77:GBT-A1, GAD-CN1c and YEp352, 78:GBT-A1, GAD-CN1c and B1/YEp352, 79:GBT-A2, GAD-CN1c and YEp352, 80:GBT-A2, GAD-CN1c and B1/YEp352, 81:GBT, GAD-CN1c and B1/YEp352, 82:GBT-A1 $\Delta$ C, GAD-CN1c and B1/YEp352, and 83:GBT-A2 $\Delta$ C, GAD-CN1c and B1/YEp352.

20 The data presented in Figures 6A and 6C are essentially equivalent to those presented in Figures 4A and 4C, respectively. The constructs and yeast strains at corresponding locations were the same. As expected, the  $\beta$ -gal signal was also essentially equivalent between the two sets. Data shown in Figure 6E demonstrate that like FK506, the immunosuppressant cyclosporin A was also effective in enhancing interaction of CN1c with CNA and CNA $\Delta$ C. 25 Both FK506 and cyclosporin A are known to exert their immunosuppressive effects through inhibition of calcineurin activity (Cyert).

Similarly, data shown in Figures 7A and 7C are essentially equivalent to those in Figures 5A and 5C, except that a top panel has been added in Figs. 7A-H. As above, the corresponding panels show the same constructs and yeast strains. The added panels (75 and 30 76) assessed the interaction of an FK506 binding protein (FKBP) with CN1c, and indicate that there were no detectable interactions between these proteins. Results in Fig. 7E demonstrate that cyclosporin A had a similar effect to FK506 in cells overexpressing CNB1 – that is, it enhanced the interactions between CN1c and CNA/CNA $\Delta$ C.

In contrast, data presented in Fig. 7G show that the immunosuppressant rapamycin, which is known not to target calcineurin, had no detectable effect on CN1c-CNA/CNAΔC interactions (compare Fig. 7G with Fig. 7A).

Taken together, the above data show that like FK506, cyclosporin A (CsA), but not rapamycin, also enhanced the interaction of CN1c with CNA and CNAΔC. CN1c didn't interact with FKBP with or without FK506.

#### EXAMPLE 4

##### Effects of CNI on FKBP/FK506 binding to Calcineurin

Y526 cells, carrying the plasmids indicated below, were grown in -Trp-Leu-His liquid media with or without FK506 (1 μg/ml) until OD<sub>600</sub> reached about 1.0. Approximately the same number of cells, calculated based on OD<sub>600</sub> and equivalent to 1 ml of an OD<sub>600</sub>=1 suspension, was harvested from each culture, washed once with ddH<sub>2</sub>O, centrifuged briefly, and the pellet was resuspended in 30 μl ddH<sub>2</sub>O and transferred onto a nitrocellulose filter. The filters were frozen in liquid nitrogen as described above, placed in a 8.5 cm petri dish containing a sheet of Whatman No. 1 paper (Whatman International LTD, Maidstone, UK) in 1.6 ml Z buffer containing 0.1% X-Gal, and incubated at 30°C for 8 hours.

Figure 8A presents exemplary data from studies to assess the effect of CNI overexpression on FK506-mediated FKBP interactions with CNA2. The legend for Fig. 8A is shown in Figure 8B. Locations of yeast colonies expressing specific constructs: 84:GBT-FKBP, GAD-A2 and YEp352, 85:GBT-FKBP, GAD-A2 and YEp352, 86:GBT-FKBP, GAD-A2 and CNI/YEp352, and 87:GBT-FKBP, GAD-A2 and CNI/YEp352. The cells at 85 and 87 were exposed to FK506, while those at 84 and 86 were not.

Data in Figure 8A demonstrate that, in the absence of FK506, FKBP and CNA2 showed no detectable interaction (84). In the presence of FK506, however, the proteins interacted (85), presumably because FK506 formed a complex with FKBP, which then bound CNA2 (Cyert).

Data in Figure 8A further show that, in the absence of FK506, CNI had no effect on the lack of interaction between FKBP and CNA2 (86). In the presence of FK506 (87), however, CNI potentiated, or enhanced the binding between FKBP and CNA2 (compare the intensity at 87 with that at 85). This effect suggests that CNI and similar compounds may be employed to increase the sensitivity of calcineurin to immunosuppressant drugs that act on it, and in this way, decrease the amount of the immunosuppressant required for a particular level of immunosuppression.

Experiments illustrated in Figures 9A and 9B demonstrate that CNI overproduction had little or no effect on the binding of CNA2 to CNB1, providing support for the specificity of the stimulatory effect that CNI overproduction had on the FK506-dependent binding of FKBP to calcineurin. The legends for Fig. 9A and 9B are shown in Figures 9B and 9D, respectively.

- 5 Locations of yeast colonies expressing the following constructs: 88:GBT-A2, GAD-B1 and YEp352, 89:GBT-A2, GAD-B1 and YEp352, 90:GBT-A2, GAD-B1 and CNI/YEp352, and 91:GBT-A2, GAD-B1 and CNI/YEp352. The colonies at 89 and 91 were exposed to FK506, while colonies at 88 and 90 were not.

- Taken together, the above data show that overexpression of the full-length CNI clone  
10 markedly enhanced the FK506-dependent interaction of FKBP with CNA, although it didn't affect the interaction between CNA and CNB1.

#### EXAMPLE 5

##### Co-Immunoprecipitation of CN1c and CNA

- 15 Yeast BJ2407 harboring AS-CN1c, which carries an influenza hemagglutinin (HA) epitope tag (Wilson, *et al.*), and GAD-A2 (lanes 1, 3) or A2/YEp352 (lanes 2, 5), and strain MCY300-1 (*cna1<sup>-</sup>cna2<sup>-</sup>*; lane 4) were grown in selective media to  $OD_{600}=0.8$ . The cells were harvested, lysed, and immunoprecipitated in the presence of 25  $\mu$ g/ml FK506 with anti-HA monoclonal antibody (obtained from M. Kirschner, Harvard University, Boston, MA; Wilson,  
20 *et al.*), following protocols described in Harlow, *et al.* The cell extracts (lanes 3-5) and the immune complex (lanes 1, 2) were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli) followed by western blot with a rabbit anti-CNA2 polyclonal antibody generated using standard methods (Harlow, *et al.*). Bound anti-CNA2 antibody was visualized with the "ECL" kit (Amersham, Arlington Heights, IL) using goat anti-  
25 rabbit antibody. Molecular weight markers are indicated on the right in kD.

The results demonstrate that CN1c was capable of binding to CNA2 tightly enough for the complex to be co-immunoprecipitated. This independent, biochemical assay confirmed the results described above obtained using the two hybrid protein interaction assay – that is, that CN1c physically interacted with and bound CNA subunits.

- 30 Cell extracts of BJ2407 harboring AS-CN1c, and GAD-A2 or A1/Yep351, and Y153b1 harboring AS-CN1c and GAD-A1 were subjected to SDS-PAGE followed by western blot with anti HA antibody. The results showed that CN1c was present at very low levels *in vivo*.

The results are consistent with the observations that a limited amount of CNA2 was precipitated by anti-HA antibody recognizing the CNic fusions, and that CNI contains PEST-like motifs, a feature of proteins with a short half-life *in vivo* (Rogers, *et al.*).

5

#### EXAMPLE 6

##### Northern Blot of CNic

Norther blots (*e.g.*, Sambrook, *et al.*) of yeast total RNA were hybridized with a CNic probe. Exemplary data are shown in Figure 11. 20  $\mu$ g yeast RNA from YPH499 (lane 1) and MCY300-1 (lane 2) was resolved in a formaldehyde-agarose gel, transferred onto "HYBOND  
10 N+" membrane (Amersham, Arlington Heights, IL), and hybridized with  $5 \times 10^6$  cpm/ml probe of the CNic insert (1.22kb). A single message of approximately 2.9 kb was detected in both strains at about the same level following an 18-hour exposure on XAR5 film (Eastman Kodak, Rochester, NY).

The data indicate that CNI was a physiologically expressed gene encoding a 2.9 kb  
15 message in yeast.

#### EXAMPLE 7

##### Chromosome Mapping of CNic

A yeast chromosome blot obtained from the ATCC was hybridized with probe of the  
20 CNic insert following the Southern hybridization procedure described in Sambrook, *et al.* A positive hybridization signal was obtained with two ATCC yeast genomic  $\lambda$  clones derived from chromosome 11. Clone 70500 had a relatively strong signal, while clone 70590 had a somewhat weaker one. A phage lysates of clone 70500 was ordered from the ATCC, amplified, purified, restriction-mapped, and used as a DNA source for cloning full length CNI  
25 (Example 1).

#### EXAMPLE 8

##### CNI null Mutants

###### 1. Construction of *cni* Null Mutation

30 A 5', 1.8 kb *Bgl*II-*Hind*III and a 3', 0.9 kb *Xba*I-*Bgl*II fragment of CNI were ligated into pRS305(LEU2) (Sikorski, *et al.*). The resultant plasmid had a deletion of a 2 kb *Hind*III-*Xba*I fragment from the coding sequence of CNI. This *cni::LEU2* mutant was introduced into the genomes of yeast haploid strains YPH499 (Sikorski, *et al.*), MCY300-1 (*cna1<sup>-</sup> cna2<sup>-</sup>*) and DD12 (*cnb1<sup>-</sup>*) (Cyert, *et al.*, 1991, Cyert, *et al.*, 1992) as well as two diploid strains.

Leucine prototrophs were isolated at high frequency from all strains, and hybridization analysis confirmed that the *cni::LEU2* allele had replaced the CNI gene. The experiments indicate that CNI is not essential for viability, since CNI deletion mutant strains (even *cni*<sup>-</sup> *cn*<sup>-</sup> double mutants) can survive.

5        CNI was deleted from three yeast strains: YPH499 (WT), MCY300-1 (*cna1*<sup>-</sup> *cna2*<sup>-</sup>), and DD12 (*cnb1*<sup>-</sup>), resulting in *cni*<sup>-</sup> strains LHy499, LHy300 and LHy12, respectively. Cells representing four colonies of each *cni* knockout strain and two colonies of each parent strain were grown in liquid YPD (Sherman, *et al.*) to saturation. Same numbers of cells from each culture were then plated onto YPD + Hygromycin B (40 µg/ml) and growth was monitored at  
10    30°C.

CNI deletions in each strain rendered that strain more resistant to hygromycin B. The effect was particularly pronounced in both MCY300-1 and DD12, suggesting that CNI functions as a suppressor of CN mutant's sensitivity to hygromycin B. The data indicate that deletion of CNI results in higher resistance to hygromycin B.

15

#### EXAMPLE 9

##### Isolation of CNI/ $\beta$ -Galactosidase Fusion Protein

A CNI coding sequence is cloned into the  $\lambda$  gt11 vector (Stratagene, La Jolla, CA). The coding frame is cloned in-frame to the  $\beta$ -galactosidase coding sequences present in  $\lambda$  gt11.  
20    Bacterial lysogens infected either with lambda phage gt11 or with gt11/CNI are incubated in 32°C until the culture reaches to an O.D. of 0.4. Then the culture is incubated in a 43°C water bath for 15 minutes to induce gt11 peptide synthesis, and further incubated at 37°C for 1 hour. Bacterial cells are pelleted and lysed in lysis buffer (10 mM Tris, pH 7.4, 2 % "TRITON X-100" and 1% aprotinin). Bacterial lysates are clarified by centrifugation (10K,  
25    for 10 minutes, Sorvall JA20 rotor) and the clarified lysates are incubated with Sepharose 4B beads conjugated with anti- $\beta$ -galactosidase (Promega).

Binding and elution of  $\beta$ -galactosidase fusion proteins are performed according to the manufacturer's instruction. Typically binding of the proteins and washing of the column are done with lysis buffer. Bound proteins are eluted with 0.1 M carbonate/bicarbonate buffer,  
30    pH 10.

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: The Board of Trustees of the Leland Stanford  
Junior University
- (ii) TITLE OF INVENTION: Calcineurin Interacting Protein Compositions  
and Methods
- (iii) NUMBER OF SEQUENCES: 23
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Dehlinger & Associates
  - (B) STREET: 350 Cambridge Avenue, Suite 250
  - (C) CITY: Palo Alto
  - (D) STATE: CA
  - (E) COUNTRY: USA
  - (F) ZIP: 94306
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE: 23-OCT-1995
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/328,322
  - (B) FILING DATE: 24-OCT-1994
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Sholtz, Charles K.
  - (B) REGISTRATION NUMBER: P38,615
  - (C) REFERENCE/DOCKET NUMBER: 8600-0151.41
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (415) 324-0880
  - (B) TELEFAX: (415) 324-0960

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1222 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (C) INDIVIDUAL ISOLATE: Sau3AI fragment containing CN1c  
coding sequence
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..918



## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAT CAA AGT AGC AAT GTC TTC GCA TCC AAA CAG CTG GTC GCA AAC ATT Asp Gln Ser Ser Asn Val Phe Ala Ser Lys Gln Leu Val Ala Asn Ile 1 5 10 15	48
TAT AAG CCC AAT CAG ATT CCA AGA GAA TTA ACT TCT CCT CAG GCG TTA Tyr Lys Pro Asn Gln Ile Pro Arg Glu Leu Thr Ser Pro Gln Ala Leu 20 25 30	96
CCA TTA TCG CCC ATC ACC TCA CCA ATT CTC AAT TAC CAA CCA TTA TCA Pro Leu Ser Pro Ile Thr Ser Pro Ile Leu Asn Tyr Gln Pro Leu Ser 35 40 45	144
AAC TCC CCG CCT CCA GAT TTT GAT TTT GAT CTA GCT AAG CGC GGC GCA Asn Ser Pro Pro Pro Asp Phe Asp Phe Asp Leu Ala Lys Arg Gly Ala 50 55 60	192
GCC GAT TCT CAT GCT ATT CCT GTG GAT CCT CCA TCA TAT TTT GAT GTA Ala Asp Ser His Ala Ile Pro Val Asp Pro Pro Ser Tyr Phe Asp Val 65 70 75 80	240
TTA AAG GCC GAT GGG ATT GAA TTG CCA TAC TAC GAT ACA AGT TCA TCT Leu Lys Ala Asp Gly Ile Glu Leu Pro Tyr Tyr Asp Thr Ser Ser Ser 85 90 95	288
AAA ATT CCT GAA CTA AAA CTA AAC AAA TCT AGA GAG ACA TTG GCC AGC Lys Ile Pro Glu Leu Lys Leu Asn Lys Ser Arg Glu Thr Leu Ala Ser 100 105 110	336
ATT GAG GAG GAC TCA TTC AAT GGT TGG TCT CAA ATT GAT GAC TTA TCC Ile Glu Glu Asp Ser Phe Asn Gly Trp Ser Gln Ile Asp Asp Leu Ser 115 120 125	384
GAC GAA GAT GAC AAT GAT GGC GAT ATA GCA TCT GGT TTC AAC TTC AAG Asp Glu Asp Asp Asn Asp Gly Asp Ile Ala Ser Gly Phe Asn Phe Lys 130 135 140	432
CTG TCA ACC AGT GCT CCG AGT GAG AAC GTT AAT TCA CAC ACT CCT ATT Leu Ser Thr Ser Ala Pro Ser Glu Asn Val Asn Ser His Thr Pro Ile 145 150 155 160	480
TTG CAG TCT TTA AAC ATG AGT CTT GAT GGG AGA AAA AAA AAT CGT GCC Leu Gln Ser Leu Asn Met Ser Leu Asp Gly Arg Lys Lys Asn Arg Ala 165 170 175	528
AGT CTA CAC GCA ACA TCA GTG TTA CCT AGT ACA ATA AGA CAG AAC AAT Ser Leu His Ala Thr Ser Val Leu Pro Ser Thr Ile Arg Gln Asn Asn 180 185 190	576
CAG CAT TTC AAT GAC ATA AAC CAG ATG CTA GGC AGT AGT GAC GAA GAT Gln His Phe Asn Asp Ile Asn Gln Met Leu Gly Ser Ser Asp Glu Asp 195 200 205	624
GCC TTT CCC AAA AGC CAA TCA TTA AAT TTC AAT AAG AAA CTA CCA ATA Ala Phe Pro Lys Ser Gln Ser Leu Asn Phe Asn Lys Lys Leu Pro Ile 210 215 220	672
CTT AAA ATT AAT GAT AAC GTC ATA CAA TCA AAC AGC AAT AGT AAT AAC Leu Lys Ile Asn Asp Asn Val Ile Gln Ser Asn Ser Asn Ser Asn Asn 225 230 235 240	720
AGA GTT GAT AAT CCA GAA GAT ACA GTG GAT TCT TCA GTC GAT ATT ACA Arg Val Asp Asn Pro Glu Asp Thr Val Asp Ser Ser Val Asp Ile Thr 245 250 255	768

40

GCA TTT TAT GAT CCA AGA ATG TCA TCA GAT TCC AAA TTT GAT TGG GAG	816
Ala Phe Tyr Asp Pro Arg Met Ser Ser Asp Ser Lys Phe Asp Trp Glu	
260 265 270	
GTA AGC AAG AAC CAT GTT GAC CCA GCA GCC TAC TCG GTT AAC GTT GCT	864
Val Ser Lys Asn His Val Asp Pro Ala Ala Tyr Ser Val Asn Val Ala	
275 280 285	
AGT GAA AAC CGT GTA CTG GAC GAC TTT AAG AAA GCA TTT CGC GAA AAG	912
Ser Glu Asn Arg Val Leu Asp Asp Phe Lys Lys Ala Phe Arg Glu Lys	
290 295 300	
AGA AAA TAAGTACATT ATTTTCATTC TCCGACAGAA TTGCTACCAT TTTACTTTGT	968
Arg Lys	
305	
GTCCTGTGAT TCAATAGTGT ACAATATATT GGACATTTTA TAGTATACAA ATATACACCA	1028
TCAATCTATA CATCCATATC ACTTGTCGTA AAGATATCCC TTTTAAATAG TACAGCGATT	1088
AAAAAATAA CATGATTAAC GTTCAGTTAC CAATGAGCTT ATTTATTAGG CTTGCTTTAG	1148
ATTTTTCCAA GTCAATTTTT GTTTTTTCTA ACGCTTGCAA CCTCATCTCA ACCTTCTTCC	1208
TTTGCAAGCA GATC	1222

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp	Gln	Ser	Ser	Asn	Val	Phe	Ala	Ser	Lys	Gln	Leu	Val	Ala	Asn	Ile
1				5				10						15	
Tyr	Lys	Pro	Asn	Gln	Ile	Pro	Arg	Glu	Leu	Thr	Ser	Pro	Gln	Ala	Leu
			20					25					30		
Pro	Leu	Ser	Pro	Ile	Thr	Ser	Pro	Ile	Leu	Asn	Tyr	Gln	Pro	Leu	Ser
		35					40					45			
Asn	Ser	Pro	Pro	Pro	Asp	Phe	Asp	Phe	Asp	Leu	Ala	Lys	Arg	Gly	Ala
	50					55					60				
Ala	Asp	Ser	His	Ala	Ile	Pro	Val	Asp	Pro	Pro	Ser	Tyr	Phe	Asp	Val
	65				70				75						80
Leu	Lys	Ala	Asp	Gly	Ile	Glu	Leu	Pro	Tyr	Tyr	Asp	Thr	Ser	Ser	Ser
			85					90						95	
Lys	Ile	Pro	Glu	Leu	Lys	Leu	Asn	Lys	Ser	Arg	Glu	Thr	Leu	Ala	Ser
		100					105						110		
Ile	Glu	Glu	Asp	Ser	Phe	Asn	Gly	Trp	Ser	Gln	Ile	Asp	Asp	Leu	Ser
		115					120					125			
Asp	Glu	Asp	Asp	Asn	Asp	Gly	Asp	Ile	Ala	Ser	Gly	Phe	Asn	Phe	Lys
	130					135					140				
Leu	Ser	Thr	Ser	Ala	Pro	Ser	Glu	Asn	Val	Asn	Ser	His	Thr	Pro	Ile
	145				150					155					160

41

Leu Gln Ser Leu Asn Met Ser Leu Asp Gly Arg Lys Lys Asn Arg Ala  
 165 170 175  
 Ser Leu His Ala Thr Ser Val Leu Pro Ser Thr Ile Arg Gln Asn Asn  
 180 185 190  
 Gln His Phe Asn Asp Ile Asn Gln Met Leu Gly Ser Ser Asp Glu Asp  
 195 200 205  
 Ala Phe Pro Lys Ser Gln Ser Leu Asn Phe Asn Lys Lys Leu Pro Ile  
 210 215 220  
 Leu Lys Ile Asn Asp Asn Val Ile Gln Ser Asn Ser Asn Ser Asn Asn  
 225 230 235 240  
 Arg Val Asp Asn Pro Glu Asp Thr Val Asp Ser Ser Val Asp Ile Thr  
 245 250 255  
 Ala Phe Tyr Asp Pro Arg Met Ser Ser Asp Ser Lys Phe Asp Trp Glu  
 260 265 270  
 Val Ser Lys Asn His Val Asp Pro Ala Ala Tyr Ser Val Asn Val Ala  
 275 280 285  
 Ser Glu Asn Arg Val Leu Asp Asp Phe Lys Lys Ala Phe Arg Glu Lys  
 290 295 300  
 Arg Lys  
 305

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 918 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: CINC coding sequence

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCAAAGTA GCAATGTCTT CGCATCCAAA CAGCTGGTCG CAAACATTTA TAAGCCCAAT	60
CAGATTCCAA GAGAATTAAC TTCTCCTCAG GCGTTACCAT TATCGCCCAT CACCTCACCA	120
ATTCTCAATT ACCAACCATT ATCAAACCTCC CCGCCTCCAG ATTTTGATTT TGATCTAGCT	180
AAGCGCGGCG CAGCCGATTC TCATGCTATT CCTGTGGATC CTCCATCATA TTTTGATGTA	240
TTAAAGGCCG ATGGGATTGA ATTGCCATAC TACGATACAA GTTCATCTAA AATTCCTGAA	300
CTAAAACTAA ACAAATCTAG AGAGACATTG GCCAGCATTG AGGAGGACTC ATTCAATGGT	360
TGGTCTCAAA TTGATGACTT ATCCGACGAA GATGACAATG ATGGCGATAT AGCATCTGGT	420
TTCAACTTCA AGCTGTCAAC CAGTGCTCCG AGTGAGAACG TTAATTCACA CACTCCTATT	480
TTGCAGTCTT TAAACATGAG TCTTGATGGG AGAAAAAATA ATCGTGCCAG TCTACACGCA	540

42

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ACATCAGTGT TACCTAGTAC AATAAGACAG AACAAATCAGC ATTTCAATGA CATAAACCAG      600
ATGCTAGGCA GTAGTGACGA AGATGCCTTT CCAAAAAGCC AATCATTAAA TTTCAATAAG      660
AAACTACCAA TACTTAAAAT TAATGATAAC GTCATACAAT CAAACAGCAA TAGTAATAAC      720
AGAGTTGATA ATCCAGAAGA TACAGTGGAT TCTTCAGTCG ATATTACAGC ATTTTATGAT      780
CCAAGAATGT CATCAGATTC CAAATTTGAT TGGGAGGTAA GCAAGAACCA TGTTGACCCA      840
GCAGCCTACT CGGTTAACGT TGCTAGTGAA AACCGTGTAC TGGACGACTT TAAGAAAGCA      900
TTTCGCGAAA AGAGAAAA                                     918

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## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3500 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (C) INDIVIDUAL ISOLATE: genomic DNA fragment containing full CNI coding sequence
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 376..3120

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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CCGAACACTT CCTTCGAGAG AGTGCATTTT ACTATGTGAA CCAATTTTTC CTCTTTTTCG      60
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AGGACTTTGC CAAATGTATT CCCGGCTGTT TGTAGTATAT ATACGCAGAT ATATAATAGC      180
GCCGTCTTTT TACCTCTTTG AGCGAATTGC CAAATATTGA CTCTTTTGTC TTATTTTCGCT      240
ATCCCCATCT TATCAAAAAT GGAACAACCT CGTTGAAATA AGAGACAAGC AACAAGAAAG      300
ACAACCAACA GAAAGTTCCA TTCCGCACAA ATACGCTGGA ATCCCATAGA ATATTGCTTG      360
TTCCTCTATG ACTAC ATG CTC CAA TTC AAT ACA GAA AAT GAT ACT GTA GCT      411
          Met Leu Gln Phe Asn Thr Glu Asn Asp Thr Val Ala
            1              5              10

CCA GTG TTT CCC ATG GAG CAA GAT ATA AAT GCA GCA CCT GAT GCC GTC      459
Pro Val Phe Pro Met Glu Gln Asp Ile Asn Ala Ala Pro Asp Ala Val
          15              20              25

CCA CTG GTG CAG ACA ACA ACA CTA CAA GTC TTT GTA AAG CTT GCC GAA      507
Pro Leu Val Gln Thr Thr Thr Leu Gln Val Phe Val Lys Leu Ala Glu
          30              35              40

CCC ATA GTG TTT TTA AAA GGA TTT GAA ACT AAC GGA CTG TCT GAA ATA      555
Pro Ile Val Phe Leu Lys Gly Phe Glu Thr Asn Gly Leu Ser Glu Ile
          45              50              55              60

```

43

GCC CCC AGT ATC TTA CGA GGA TCT CTT ATC GTC AGG GTG TTG AAA CCG Ala Pro Ser Ile Leu Arg Gly Ser Leu Ile Val Arg Val Leu Lys Pro 65 70 75	603
AAT AAA TTA AAA AGT ATA TCG ATA ACC TTC AAA GGA ATA TCC AGA ACA Asn Lys Leu Lys Ser Ile Ser Ile Thr Phe Lys Gly Ile Ser Arg Thr 80 85 90	651
GAG TGG CCG GAA GGT ATA CCA CCG AAG AGA GAA GAA TTT TCA GAT GTT Glu Trp Pro Glu Gly Ile Pro Pro Lys Arg Glu Glu Phe Ser Asp Val 95 100 105	699
GAA ACT GTT GTC AAT CAC ACA TGG CCA TTT TAT CAG GCG GAT GAC GGC Glu Thr Val Val Asn His Thr Trp Pro Phe Tyr Gln Ala Asp Asp Gly 110 115 120	747
ATG AAT TCT TTC ACC TTA GAA CAT CAC AGC TCA AAT AAT TCG TCC AAT Met Asn Ser Phe Thr Leu Glu His His Ser Ser Asn Asn Ser Ser Asn 125 130 135 140	795
CGC CCA TCT ATG AGC GAT GAA GAT TAT CTA CTT GAA AAA AGC GGT GCT Arg Pro Ser Met Ser Asp Glu Asp Tyr Leu Leu Glu Lys Ser Gly Ala 145 150 155	843
TCA GTA TAT ATC CCA CCA ACC GCT GAA CCC CCT AAA GAT AAT AGC AAT Ser Val Tyr Ile Pro Pro Thr Ala Glu Pro Pro Lys Asp Asn Ser Asn 160 165 170	891
CTA AGT CTG GAT GCC TAT GAG CGC AAC TCA TTG TCA TCC GAT AAT TTG Leu Ser Leu Asp Ala Tyr Glu Arg Asn Ser Leu Ser Ser Asp Asn Leu 175 180 185	939
AGT AAC AAG CCA GTA TCA AGT GAT GTT TCC CAT GAC GAC AGT AAA CTG Ser Asn Lys Pro Val Ser Ser Asp Val Ser His Asp Asp Ser Lys Leu 190 195 200	987
TTG GCT ATT CAA AAG ACA CCA TTA CCA TCA TCT AGT CGA AGA GGA TCG Leu Ala Ile Gln Lys Thr Pro Leu Pro Ser Ser Ser Arg Arg Gly Ser 205 210 215 220	1035
GTA CCG GCA AAT TTT CAC GGT AAC TCT TTG TCA CCT CAT ACC TTC ATA Val Pro Ala Asn Phe His Gly Asn Ser Leu Ser Pro His Thr Phe Ile 225 230 235	1083
TCT GAT TTG TTC ACA AAA ACA TTC AGT AAT AGT GGC GCT ACT CCA AGT Ser Asp Leu Phe Thr Lys Thr Phe Ser Asn Ser Gly Ala Thr Pro Ser 240 245 250	1131
CCT GAG CAA GAG GAT AAC TAT CTT ACA CCA TCC AAA GAT TCT AAA GAA Pro Glu Gln Glu Asp Asn Tyr Leu Thr Pro Ser Lys Asp Ser Lys Glu 255 260 265	1179
GTT TTT ATT TTT CGA CCG GGC GAT TAT ATT TAC ACT TTT GAA CAG CCA Val Phe Ile Phe Arg Pro Gly Asp Tyr Ile Tyr Thr Phe Glu Gln Pro 270 275 280	1227
ATA TCG CAA TCT TAT CCA GAA AGT ATA AAA GCC AAT TTT GGT TCC GTG Ile Ser Gln Ser Tyr Pro Glu Ser Ile Lys Ala Asn Phe Gly Ser Val 285 290 295 300	1275
GAG TAT AAA CTG TCA ATA GAC ATA GAG AGG TTT GGC GCA TTC AAA TCA Glu Tyr Lys Leu Ser Ile Asp Ile Glu Arg Phe Gly Ala Phe Lys Ser 305 310 315	1323
ACT ATA CAT ACT CAA TTA CCC ATC AAA GTC GTA AGG CTT CCT TCT GAT Thr Ile His Thr Gln Leu Pro Ile Lys Val Val Arg Leu Pro Ser Asp 320 325 330	1371

GGA TCC GTA GAA GAG ACT GAA GCT ATT GCA ATT TCC AAG GAC TGG AAA Gly Ser Val Glu Glu Thr Glu Ala Ile Ala Ile Ser Lys Asp Trp Lys 335 340 345	1419
GAT CTT CTT CAT TAT GAC GTG GTA ATT TTC TCG AAA GAG ATC GTT TTG Asp Leu Leu His Tyr Asp Val Val Ile Phe Ser Lys Glu Ile Val Leu 350 355 360	1467
AAT GCA TTT TTA CCC ATC GAT TTC CAT TTC GCT CCT CTA GAT AAA GTT Asn Ala Phe Leu Pro Ile Asp Phe His Phe Ala Pro Leu Asp Lys Tyr Val 365 370 375 380	1515
ACT CTG CAT CGT ATT AGA ATT TAT CTA ACA GAG TCT ATG GAA TAC ACT Thr Leu His Arg Ile Arg Ile Tyr Leu Thr Glu Ser Met Glu Tyr Thr 385 390 395	1563
TGT AAT AGT AAT GGA AAT CAC GAG AAG GCT CGT AGA TTA GAG CCA ACT Cys Asn Ser Asn Gly Asn His Glu Lys Ala Arg Arg Leu Glu Pro Thr 400 405 410	1611
AAA AAG TTT CTG TTG GCT GAA CAT AAC GGT CCT AAA CTG CCT CAT ATA Lys Lys Phe Leu Leu Ala Glu His Asn Gly Pro Lys Leu Pro His Ile 415 420 425	1659
CCA GCT GGT TCG AAT CCT TTG AAG GCT AAA AAT AGA GGG AAC ATC CTC Pro Ala Gly Ser Asn Pro Leu Lys Ala Lys Asn Arg Gly Asn Ile Leu 430 435 440	1707
TTG GAT GAA AAA TCC GGC GAT CTA GTT AAC AAA GAT TTT CAG TTC GAG Leu Asp Glu Lys Ser Gly Asp Leu Val Asn Lys Asp Phe Gln Phe Glu 445 450 455 460	1755
GTG TTT GTC CCA AGC AAG TTT ACA AAC AGT ATA CGG TTA CAC CCT GAT Val Phe Val Pro Ser Lys Phe Thr Asn Ser Ile Arg Leu His Pro Asp 465 470 475	1803
ACA AAT TAT GAT AAA ATC AAA GCC CAC CAT TGG ATA AAA ATT TGC CTT Thr Asn Tyr Asp Lys Ile Lys Ala His His Trp Ile Lys Ile Cys Leu 480 485 490	1851
CGT CTT TCC AAG AAG TAC GGG GAC AAT AGA AAA CAT TTC GAA ATA AGT Arg Leu Ser Lys Lys Tyr Gly Asp Asn Arg Lys His Phe Glu Ile Ser 495 500 505	1899
ATT GAT TCT CCA ATC CAT ATT TTA AAT CAA CTA TGC TCA CAC GCG AAT Ile Asp Ser Pro Ile His Ile Leu Asn Gln Leu Cys Ser His Ala Asn 510 515 520	1947
ACT TTG CTA CCG AGC TAC GAG AGT CAT TTC CAG TAT TGT GAT GAA GAT Thr Leu Leu Pro Ser Tyr Glu Ser His Phe Gln Tyr Cys Asp Glu Asp 525 530 535 540	1995
GGT AAT TTC GCA CCA GCA GCA GAT CAA CAA AAT TAC GCA AGT CAT CAT Gly Asn Phe Ala Pro Ala Ala Asp Gln Gln Asn Tyr Ala Ser His His 545 550 555	2043
GAT TCC AAT ATT TTC TTC CCA AAA GAA GTT CTT TCG TCT CCC GTT CTT Asp Ser Asn Ile Phe Phe Pro Lys Glu Val Leu Ser Ser Pro Val Leu 560 565 570	2091
TCA CCT AAC GTG CAG AAG ATG AAC ATT AGA ATA CCG TCT GAT CTT CCA Ser Pro Asn Val Gln Lys Met Asn Ile Arg Ile Pro Ser Asp Leu Pro 575 580 585	2139
GTA GTG CGT AAT AGA GCT GAA AGC GTA AAG AAA AGC AAG TCA GAT AAT Val Val Arg Asn Arg Ala Glu Ser Val Lys Lys Ser Lys Ser Asp Asn 590 595 600	2187

45

ACC	TCC	AAG	AAG	AAT	GAT	CAA	AGT	AGC	AAT	GTC	TTC	GCA	TCC	AAA	CAG	2235
Thr	Ser	Lys	Lys	Asn	Asp	Gln	Ser	Ser	Asn	Val	Phe	Ala	Ser	Lys	Gln	
605					610					615					620	
CTG	GTC	GCA	AAC	ATT	TAT	AAG	CCC	AAT	CAG	ATT	CCA	AGA	GAA	TTA	ACT	2283
Leu	Val	Ala	Asn	Ile	Tyr	Lys	Pro	Asn	Gln	Ile	Pro	Arg	Glu	Leu	Thr	
				625					630						635	
TCT	CCT	CAG	GCG	TTA	CCA	TTA	TCG	CCC	ATC	ACC	TCA	CCA	ATT	CTC	AAT	2331
Ser	Pro	Gln	Ala	Leu	Pro	Leu	Ser	Pro	Ile	Thr	Ser	Pro	Ile	Leu	Asn	
			640					645							650	
TAC	CAA	CCA	TTA	TCA	AAC	TCC	CCG	CCT	CCA	GAT	TTT	GAT	TTT	GAT	CTA	2379
Tyr	Gln	Pro	Leu	Ser	Asn	Ser	Pro	Pro	Pro	Asp	Phe	Asp	Phe	Asp	Leu	
		655					660					665				
GCT	AAG	CGC	GGC	GCA	GCC	GAT	TCT	CAT	GCT	ATT	CCT	GTG	GAT	CCT	CCA	2427
Ala	Lys	Arg	Gly	Ala	Ala	Asp	Ser	His	Ala	Ile	Pro	Val	Asp	Pro	Pro	
	670					675					680					
TCA	TAT	TTT	GAT	GTA	TTA	AAG	GCC	GAT	GGG	ATT	GAA	TTG	CCA	TAC	TAC	2475
Ser	Tyr	Phe	Asp	Val	Leu	Lys	Ala	Asp	Gly	Ile	Glu	Leu	Pro	Tyr	Tyr	
685					690				695						700	
GAT	ACA	AGT	TCA	TCT	AAA	ATT	CCT	GAA	CTA	AAA	CTA	AAC	AAA	TCT	AGA	2523
Asp	Thr	Ser	Ser	Ser	Lys	Ile	Pro	Glu	Leu	Lys	Leu	Asn	Lys	Ser	Arg	
				705					710						715	
GAG	ACA	TTG	GCC	AGC	ATT	GAG	GAG	GAC	TCA	TTC	AAT	GGT	TGG	TCT	CAA	2571
Glu	Thr	Leu	Ala	Ser	Ile	Glu	Glu	Asp	Ser	Phe	Asn	Gly	Trp	Ser	Gln	
			720					725							730	
ATT	GAT	GAC	TTA	TCC	GAC	GAA	GAT	GAC	AAT	GAT	GGC	GAT	ATA	GCA	TCT	2619
Ile	Asp	Asp	Leu	Ser	Asp	Glu	Asp	Asp	Asn	Asp	Gly	Asp	Ile	Ala	Ser	
		735					740					745				
GGT	TTC	AAC	TTC	AAG	CTG	TCA	ACC	AGT	GCT	CCG	AGT	GAG	AAC	GTT	AAT	2667
Gly	Phe	Asn	Phe	Lys	Leu	Ser	Thr	Ser	Ala	Pro	Ser	Glu	Asn	Val	Asn	
	750					755					760					
TCA	CAC	ACT	CCT	ATT	TTG	CAG	TCT	TTA	AAC	ATG	AGT	CTT	GAT	GGG	AGA	2715
Ser	His	Thr	Pro	Ile	Leu	Gln	Ser	Leu	Asn	Met	Ser	Leu	Asp	Gly	Arg	
765					770					775					780	
AAA	AAA	AAT	CGT	GCC	AGT	CTA	CAC	GCA	ACA	TCA	GTG	TTA	CCT	AGT	ACA	2763
Lys	Lys	Asn	Arg	Ala	Ser	Leu	His	Ala	Thr	Ser	Val	Leu	Pro	Ser	Thr	
				785					790						795	
ATA	AGA	CAG	AAC	AAT	CAG	CAT	TTC	AAT	GAC	ATA	AAC	CAG	ATG	CTA	GGC	2811
Ile	Arg	Gln	Asn	Asn	Gln	His	Phe	Asn	Asp	Ile	Asn	Gln	Met	Leu	Gly	
			800					805							810	
AGT	AGT	GAC	GAA	GAT	GCC	TTT	CCC	AAA	AGC	CAA	TCA	TTA	AAT	TTC	AAT	2859
Ser	Ser	Asp	Glu	Asp	Ala	Phe	Pro	Lys	Ser	Gln	Ser	Leu	Asn	Phe	Asn	
		815					820								825	
AAG	AAA	CTA	CCA	ATA	CTT	AAA	ATT	AAT	GAT	AAC	GTC	ATA	CAA	TCA	AAC	2907
Lys	Lys	Leu	Pro	Ile	Leu	Lys	Ile	Asn	Asp	Asn	Val	Ile	Gln	Ser	Asn	
		830				835					840					
AGC	AAT	AGT	AAT	AAC	AGA	GTT	GAT	AAT	CCA	GAA	GAT	ACA	GTG	GAT	TCT	2955
Ser	Asn	Ser	Asn	Asn	Arg	Val	Asp	Asn	Pro	Glu	Asp	Thr	Val	Asp	Ser	
845					850					855					860	

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TCA GTC GAT ATT ACA GCA TTT TAT GAT CCA AGA ATG TCA TCA GAT TCC	3003
Ser Val Asp Ile Thr Ala Phe Tyr Asp Pro Arg Met Ser Ser Asp Ser	
865 870 875	
AAA TTT GAT TGG GAG GTA AGC AAG AAC CAT GTT GAC CCA GCA GCC TAC	3051
Lys Phe Asp Trp Glu Val Ser Lys Asn His Val Asp Pro Ala Ala Tyr	
880 885 890	
TCG GTT AAC GTT GCT AGT GAA AAC CGT GTA CTG GAC GAC TTT AAG AAA	3099
Ser Val Asn Val Ala Ser Glu Asn Arg Val Leu Asp Asp Phe Lys Lys	
895 900 905	
GCA TTT CGC GAA AAG AGA AAA TAAGTACATT ATTTTCATT CCGACAGAA	3150
Ala Phe Arg Glu Lys Arg Lys	
910 915	
TTGCTACCAT TTTACTTTGT GTCCTGTGAT TCAATAGTGT ACAATATATT GGACATTTTA	3210
TAGTATACAA ATATACACCA TCAATCTATA CATCCATATC ACTTGTCGTA AAGATATCCC	3270
TTTTTAATAG TACAGCGATT AAAAAAATAA CATGATTAAC GTTCAGTTAC CAATGAGCTT	3330
ATTTATTAGG CTTGCTTTAG ATTTTCCAA GTCAATTTTT GTTTTTTCTA ACGCTTGCAA	3390
CCTCATCTCA ACCTTCTTCC TTTGCAAGCA GATCTTCGAA ACCATCTCGT TTATTCTCTC	3450
AATGCTGTTT CCACTTTCAT CATCGTCTGG GAAAAGTACC GGTAAGGGCG	3500

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 915 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Leu	Gln	Phe	Asn	Thr	Glu	Asn	Asp	Thr	Val	Ala	Pro	Val	Phe	Pro
1				5					10					15	
Met	Glu	Gln	Asp	Ile	Asn	Ala	Ala	Pro	Asp	Ala	Val	Pro	Leu	Val	Gln
			20					25					30		
Thr	Thr	Thr	Leu	Gln	Val	Phe	Val	Lys	Leu	Ala	Glu	Pro	Ile	Val	Phe
			35				40					45			
Leu	Lys	Gly	Phe	Glu	Thr	Asn	Gly	Leu	Ser	Glu	Ile	Ala	Pro	Ser	Ile
	50					55				60					
Leu	Arg	Gly	Ser	Leu	Ile	Val	Arg	Val	Leu	Lys	Pro	Asn	Lys	Leu	Lys
	65				70				75					80	
Ser	Ile	Ser	Ile	Thr	Phe	Lys	Gly	Ile	Ser	Arg	Thr	Glu	Trp	Pro	Glu
				85					90					95	
Gly	Ile	Pro	Pro	Lys	Arg	Glu	Glu	Phe	Ser	Asp	Val	Glu	Thr	Val	Val
			100					105					110		
Asn	His	Thr	Trp	Pro	Phe	Tyr	Gln	Ala	Asp	Asp	Gly	Met	Asn	Ser	Phe
		115					120					125			
Thr	Leu	Glu	His	His	Ser	Ser	Asn	Asn	Ser	Ser	Asn	Arg	Pro	Ser	Met
	130					135					140				



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Ser Asp Glu Asp Tyr Leu Leu Glu Lys Ser Gly Ala Ser Val Tyr Ile  
 145 150 155 160  
 Pr Pro Thr Ala Glu Pro Pro Lys Asp Asn Ser Asn L u Ser Leu Asp  
 165 170 175  
 Ala Tyr Glu Arg Asn Ser Leu Ser Ser Asp Asn Leu Ser Asn Lys Pro  
 180 185 190  
 Val Ser Ser Asp Val Ser His Asp Asp Ser Lys Leu Leu Ala Ile Gln  
 195 200 205  
 Lys Thr Pro Leu Pro Ser Ser Ser Arg Arg Gly Ser Val Pro Ala Asn  
 210 215 220  
 Phe His Gly Asn Ser Leu Ser Pro His Thr Phe Ile Ser Asp Leu Phe  
 225 230 235 240  
 Thr Lys Thr Phe Ser Asn Ser Gly Ala Thr Pro Ser Pro Glu Gln Glu  
 245 250 255  
 Asp Asn Tyr Leu Thr Pro Ser Lys Asp Ser Lys Glu Val Phe Ile Phe  
 260 265 270  
 Arg Pro Gly Asp Tyr Ile Tyr Thr Phe Glu Gln Pro Ile Ser Gln Ser  
 275 280 285  
 Tyr Pro Glu Ser Ile Lys Ala Asn Phe Gly Ser Val Glu Tyr Lys Leu  
 290 295 300  
 Ser Ile Asp Ile Glu Arg Phe Gly Ala Phe Lys Ser Thr Ile His Thr  
 305 310 315 320  
 Gln Leu Pro Ile Lys Val Val Arg Leu Pro Ser Asp Gly Ser Val Glu  
 325 330 335  
 Glu Thr Glu Ala Ile Ala Ile Ser Lys Asp Trp Lys Asp Leu Leu His  
 340 345 350  
 Tyr Asp Val Val Ile Phe Ser Lys Glu Ile Val Leu Asn Ala Phe Leu  
 355 360 365  
 Pro Ile Asp Phe His Phe Ala Pro Leu Asp Lys Val Thr Leu His Arg  
 370 375 380  
 Ile Arg Ile Tyr Leu Thr Glu Ser Met Glu Tyr Thr Cys Asn Ser Asn  
 385 390 395 400  
 Gly Asn His Glu Lys Ala Arg Arg Leu Glu Pro Thr Lys Lys Phe Leu  
 405 410 415  
 Leu Ala Glu His Asn Gly Pro Lys Leu Pro His Ile Pro Ala Gly Ser  
 420 425 430  
 Asn Pro Leu Lys Ala Lys Asn Arg Gly Asn Ile Leu Leu Asp Glu Lys  
 435 440 445  
 Ser Gly Asp Leu Val Asn Lys Asp Phe Gln Phe Glu Val Phe Val Pro  
 450 455 460  
 Ser Lys Phe Thr Asn Ser Ile Arg Leu His Pro Asp Thr Asn Tyr Asp  
 465 470 475 480  
 Lys Ile Lys Ala His His Trp Ile Lys Ile Cys Leu Arg Leu Ser Lys  
 485 490 495

Lys Tyr Gly Asp Asn Arg Lys His Phe Glu Ile Ser Ile Asp Ser Pro  
 500 505 510  
 Ile His Ile Leu Asn Gln Leu Cys Ser His Ala Asn Thr Leu Leu Pro  
 515 520 525  
 Ser Tyr Glu Ser His Phe Gln Tyr Cys Asp Glu Asp Gly Asn Phe Ala  
 530 535 540  
 Pro Ala Ala Asp Gln Gln Asn Tyr Ala Ser His His Asp Ser Asn Ile  
 545 550 555 560  
 Phe Phe Pro Lys Glu Val Leu Ser Ser Pro Val Leu Ser Pro Asn Val  
 565 570 575  
 Gln Lys Met Asn Ile Arg Ile Pro Ser Asp Leu Pro Val Val Arg Asn  
 580 585 590  
 Arg Ala Glu Ser Val Lys Lys Ser Lys Ser Asp Asn Thr Ser Lys Lys  
 595 600 605  
 Asn Asp Gln Ser Ser Asn Val Phe Ala Ser Lys Gln Leu Val Ala Asn  
 610 615 620  
 Ile Tyr Lys Pro Asn Gln Ile Pro Arg Glu Leu Thr Ser Pro Gln Ala  
 625 630 635 640  
 Leu Pro Leu Ser Pro Ile Thr Ser Pro Ile Leu Asn Tyr Gln Pro Leu  
 645 650 655  
 Ser Asn Ser Pro Pro Pro Asp Phe Asp Phe Asp Leu Ala Lys Arg Gly  
 660 665 670  
 Ala Ala Asp Ser His Ala Ile Pro Val Asp Pro Pro Ser Tyr Phe Asp  
 675 680 685  
 Val Leu Lys Ala Asp Gly Ile Glu Leu Pro Tyr Tyr Asp Thr Ser Ser  
 690 695 700  
 Ser Lys Ile Pro Glu Leu Lys Leu Asn Lys Ser Arg Glu Thr Leu Ala  
 705 710 715 720  
 Ser Ile Glu Glu Asp Ser Phe Asn Gly Trp Ser Gln Ile Asp Asp Leu  
 725 730 735  
 Ser Asp Glu Asp Asp Asn Asp Gly Asp Ile Ala Ser Gly Phe Asn Phe  
 740 745 750  
 Lys Leu Ser Thr Ser Ala Pro Ser Glu Asn Val Asn Ser His Thr Pro  
 755 760 765  
 Ile Leu Gln Ser Leu Asn Met Ser Leu Asp Gly Arg Lys Lys Asn Arg  
 770 775 780  
 Ala Ser Leu His Ala Thr Ser Val Leu Pro Ser Thr Ile Arg Gln Asn  
 785 790 795 800  
 Asn Gln His Phe Asn Asp Ile Asn Gln Met Leu Gly Ser Ser Asp Glu  
 805 810 815  
 Asp Ala Phe Pro Lys Ser Gln Ser Leu Asn Phe Asn Lys Lys Leu Pro  
 820 825 830  
 Ile Leu Lys Ile Asn Asp Asn Val Ile Gln Ser Asn Ser Asn Ser Asn  
 835 840 845

Asn Arg Val Asp Asn Pr Glu Asp Thr Val Asp Ser Ser Val Asp Ile  
 850 855 860  
 Thr Ala Phe Tyr Asp Pro Arg Met Ser Ser Asp Ser Lys Phe Asp Trp  
 865 870 875 880  
 Glu Val Ser Lys Asn His Val Asp Pro Ala Ala Tyr Ser Val Asn Val  
 885 890 895  
 Ala Ser Glu Asn Arg Val Leu Asp Asp Phe Lys Lys Ala Phe Arg Glu  
 900 905 910  
 Lys Arg Lys  
 915

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2745 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: coding sequence of CNI

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCTCCAAT TCAATACAGA AAATGATACT GTAGCTCCAG TGTTTCCCAT GGAGCAAGAT	60
ATAAATGCAG CACCTGATGC CGTCCCCTG GTGCAGACAA CAACACTACA AGTCTTTGTA	120
AAGCTTGCCG AACCCATAGT GTTTTTAAAA GGATTGAAA CTAACGGACT GTCTGAAATA	180
GCCCCAGTA TCTTACGAGG ATCTCTTATC GTCAGGGTGT TGAAACCGAA TAAATTAAAA	240
AGTATATCGA TAACCTTCAA AGGAATATCC AGAACAGAGT GGCCGGAAGG TATACCACCG	300
AAGAGAGAAG AATTTTCAGA TGTTGAACT GTTGTCATC ACACATGGCC ATTTTATCAG	360
GCGGATGACG GCATGAATTC TTTCACCTTA GAACATCACA GCTCAAATAA TTCGTCCAAT	420
CGCCCATCTA TGAGCGATGA AGATTATCTA CTTGAAAAA GCGGTGCTTC AGTATATATC	480
CCACCAACCG CTGAACCCCC TAAAGATAAT AGCAATCTAA GTCTGGATGC CTATGAGCGC	540
AACTCATTGT CATCCGATAA TTTGAGTAAC AAGCCAGTAT CAAGTGATGT TTCCCATGAC	600
GACAGTAAAC TGTTGGCTAT TCAAAAGACA CCATTACCAT CATCTAGTCG AAGAGGATCG	660
GTACCGGCAA ATTTTCACGG TAACTCTTTG TCACCTCATA CCTTCATATC TGATTTGTTC	720
ACAAAAACAT TCAGTAATAG TGGCGCTACT CCAAGTCCTG AGCAAGAGGA TAACTATCTT	780
ACACCATCCA AAGATTCTAA AGAAGTTTTT ATTTTTCGAC CGGGCGATTA TATTTACACT	840
TTTGAACAGC CAATATCGCA ATCTTATCCA GAAAGTATAA AAGCCAATTT TGGTTCCGTG	900
GAGTATAAAC TGTCAATAGA CATAGAGAGG TTTGGCGCAT TCAAATCAAC TATACATACT	960

CAATTACCCA TCAAAGTCGT AAGGCTTCCT TCTGATGGAT CCGTAGAAGA GACTGAAGCT	1020
ATTGCAATTT CCAAGGACTG GAAAGATCTT CTTCAATTATG ACGTGGTAAT TTTCTCGAAA	1080
GAGATCGTTT TGAATGCATT TTTACCCATC GATTTCCATT TCGCTCCTCT AGATAAAGTT	1140
ACTCTGCATC GTATTAGAAT TTATCTAACA GAGTCTATGG AATACACTTG TAATAGTAAT	1200
GGAATCACG AGAAGGCTCG TAGATTAGAG CCAACTAAAA AGTTTCTGTT GGCTGAACAT	1260
AACGGTCCTA AACTGCCTCA TATACCAGCT GGTTCGAATC CTTTGAAGGC TAAAAATAGA	1320
GGGAACATCC TCTTGGATGA AAAATCCGGC GATCTAGTTA ACAAAGATT TCAAGTTCGAG	1380
GTGTTTGTCC CAAGCAAGTT TACAAACAGT ATACGGTTAC ACCCTGATAC AAATTATGAT	1440
AAAATCAAAG CCCACCATTG GATAAAAATT TGCCTTCGTC TTTCCAAGAA GTACGGGGAC	1500
AATAGAAAAC ATTTCGAAAT AAGTATTGAT TCTCCAATCC ATATTTTAAA TCAACTATGC	1560
TCACACGCGA ATACTTTGCT ACCGAGCTAC GAGAGTCATT TCCAGTATTG TGATGAAGAT	1620
GGTAATTTTCG CACCAGCAGC AGATCAACAA AATTACGCAA GTCATCATGA TTCCAATATT	1680
TTCTTCCCAA AAGAAGTTCT TTCGTCTCCC GTTCTTTTAC CTAACGTGCA GAAGATGAAC	1740
ATTAGAATAC CGTCTGATCT TCCAGTAGTG CGTAATAGAG CTGAAAGCGT AAAGAAAAGC	1800
AAGTCAGATA ATACCTCCAA GAAGAATGAT CAAAGTAGCA ATGTCTTCGC ATCCAAACAG	1860
CTGGTCGCAA ACATTTATAA GCCCAATCAG ATTCCAAGAG AATTAAGTTC TCCTCAGGCG	1920
TTACCATTAT CGCCCATCAC CTCACCAATT CTCAATTACC AACCATTATC AAATCCCCG	1980
CCTCCAGATT TTGATTTTGA TCTAGCTAAG CGCGGCGCAG CCGATTCTCA TGCTATTCTT	2040
GTGGATCCTC CATCATATTT TGATGTATTA AAGGCCGATG GGATTGAATT GCCATACTAC	2100
GATACAAGTT CATCTAAAT TCCTGAAC TAACATAACA AATCTAGAGA GACATTGGCC	2160
AGCATTGAGG AGGACTCATT CAATGGTTGG TCTCAAATTG ATGACTTATC CGACGAAGAT	2220
GACAATGATG GCGATATAGC ATCTGGTTTC AACTTCAAGC TGTCAACCAG TGCTCCGAGT	2280
GAGAACGTTA ATTCACACAC TCCTATTTTG CAGTCTTTAA ACATGAGTCT TGATGGGAGA	2340
AAAAAAATC GTGCCAGTCT ACACGCAACA TCAGTGTTAC CTAGTACAAT AAGACAGAAC	2400
AATCAGCATT TCAATGACAT AAACCAGATG CTAGGCAGTA GTGACGAAGA TGCCTTTCCC	2460
AAAAGCCAAT CATTAAATTT CAATAAGAAA CTACCAATAC TTAAAATTAA TGATAACGTC	2520
ATACAATCAA ACAGCAATAG TAATAACAGA GTTGATAATC CAGAAGATAC AGTGGATTCT	2580
TCAGTCGATA TTACAGCATT TTATGATCCA AGAATGTCAT CAGATTCCAA ATTTGATTGG	2640
GAGGTAAGCA AGAACCATGT TGACCCAGCA GCCTACTCGG TTAACGTTGC TAGTGAAAAC	2700
CGTGTACTGG ACGACTTTAA GAAAGCATTT CGCGAAAAGA GAAAA	2745

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: CNI-PRC-A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCAAAAAAG AGATCTCGGA TCAAAGTAGC

30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: CNI-PCR-B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGTTTTTCA GTGTCGACGA TTCATAGATC

30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1964 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: genomic DNA fragment containing full  
CNA1 coding sequence

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 286..1944

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTTTGTTGCA TTTTGATATT CATCTATATC TATTTCAAAA TTTTCATGT CATCGCCTCT

60

TGAAACATGA ATTTTCCAAT TCTGAAAAAG AACGTACTAC TGGGAAACAA AAGGGAAAAA	120
TGTATAAATC CTTTAATGTT TTTGAATCAA GAGGCATTAT TATAAAAGAA CGAAGCAAAG	180
CCTTTAATAT TTGCTTTATT AAAGGTATTA TTCAAAGAAA AGTTTTTTTA GATTCTTTTT	240
TTTTTGACGT ATTAGCTCAG CTGCCATAAA ACACTCTCAA CGCCA ATG TCG AAA	294
Met Ser Lys	
1	
GAC TTG AAT TCT TCA CGC ATC AAA ATC ATT AAA CCT AAT GAC TCT TAC	342
Asp Leu Asn Ser Ser Arg Ile Lys Ile Ile Lys Pro Asn Asp Ser Tyr	
5 10 15	
ATA AAG GTT GAC CGG AAA AAA GAT TTA ACA AAA TAC GAA TTA GAA AAC	390
Ile Lys Val Asp Arg Lys Lys Asp Leu Thr Lys Tyr Glu Leu Glu Asn	
20 25 30 35	
GGT AAA GTA ATT TCT ACT AAG GAC CGA TCC TAC GCT TCT GTA CCT GCC	438
Gly Lys Val Ile Ser Thr Lys Asp Arg Ser Tyr Ala Ser Val Pro Ala	
40 45 50	
ATA ACA GGA AAG ATA CCA AGT GAT GAG GAA GTA TTC GAC TCC AAG ACG	486
Ile Thr Gly Lys Ile Pro Ser Asp Glu Glu Val Phe Asp Ser Lys Thr	
55 60 65	
GGA TTA CCT AAT CAT TCC TTT TTA AGA GAG CAT TTC TTT CAT GAG GGT	534
Gly Leu Pro Asn His Ser Phe Leu Arg Glu His Phe Phe His Glu Gly	
70 75 80	
CGA CTT TCT AAG GAA CAG GCC ATA AAA ATC TTA AAT ATG TCA ACT GTA	582
Arg Leu Ser Lys Glu Gln Ala Ile Lys Ile Leu Asn Met Ser Thr Val	
85 90 95	
GCA TTG AGT AAA GAA CCC AAT CTA CTA AAA CTC AAA GCG CCA ATT ACT	630
Ala Leu Ser Lys Glu Pro Asn Leu Leu Lys Leu Lys Ala Pro Ile Thr	
100 105 110 115	
ATA TGT GGT GAT ATT CAC GGG CAG TAT TAT GAT TTA TTG AAA CTG TTT	678
Ile Cys Gly Asp Ile His Gly Gln Tyr Tyr Asp Leu Leu Lys Leu Phe	
120 125 130	
GAA GTT GGC GGT GAC CCC GCC GAA ATC GAC TAT TTA TTC TTG GGG GAT	726
Glu Val Gly Gly Asp Pro Ala Glu Ile Asp Tyr Leu Phe Leu Gly Asp	
135 140 145	
TAT GTT GAT AGA GGT GCA TTC TCT TTT GAG TGT CTG ATT TAT TTG TAC	774
Tyr Val Asp Arg Gly Ala Phe Ser Phe Glu Cys Leu Ile Tyr Leu Tyr	
150 155 160	
TCC TTG AAG TTG AAT AAT TTA GGG AGA TTT TGG ATG CTA AGA GGT AAC	822
Ser Leu Lys Leu Asn Asn Leu Gly Arg Phe Trp Met Leu Arg Gly Asn	
165 170 175	
CAT GAG TGT AAG CAC TTG ACC TCT TAT TTT ACT TTT AAG AAT GAG ATG	870
His Glu Cys Lys His Leu Thr Ser Tyr Phe Thr Phe Lys Asn Glu Met	
180 185 190 195	
TTG CAC AAA TAC GAT ATG GAA GTT TAC GAT GCT TGC TGC AGA TCA TTC	918
Leu His Lys Tyr Asp Met Glu Val Tyr Asp Ala Cys Cys Arg Ser Phe	
200 205 210	
AAT GTC TTA CCA TTA GCA GCT TTA ATG AAC GGA CAA TAT TTT TGT GTG	966
Asn Val Leu Pro Leu Ala Ala Leu Met Asn Gly Gln Tyr Ph Cys Val	
215 220 225	

CAT GGT GGT ATC TCT CCA GAG TTA AAA TCA GTA GAG GAT GTT AAT AAA His Gly Gly Ile Ser Pro Glu Leu Lys Ser Val Glu Asp Val Asn Lys 230 235 240	1014
ATT AAT AGA TTT CGA GAA ATC CCA TCT CGT GGT CTC ATG TGT GAC CTA Ile Asn Arg Phe Arg Glu Ile Pro Ser Arg Gly Leu Met Cys Asp Leu 245 250 255	1062
CTA TGG GCC GAT CCT GTC GAA AAT TAT GAT GAT GCA AGA GAT GGT AGC Leu Trp Ala Asp Pro Val Glu Asn Tyr Asp Asp Ala Arg Asp Gly Ser 260 265 270 275	1110
GAA TTT GAT CAG AGC GAG GAT GAA TTC GTA CCT AAC AGT TTG AGG GGT Glu Phe Asp Gln Ser Glu Asp Glu Phe Val Pro Asn Ser Leu Arg Gly 280 285 290	1158
TGC TCT TTC GCC TTC ACT TTT AAA GCA TCA TGC AAG TTT TTG AAG GCA Cys Ser Phe Ala Phe Thr Phe Lys Ala Ser Cys Lys Phe Leu Lys Ala 295 300 305	1206
AAT GGT TTG TTA TCT ATT ATT AGA GCA CAC GAA GCA CAG GAT GCT GGG Asn Gly Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp Ala Gly 310 315 320	1254
TAC AGA ATG TAT AAA AAC AAT AAA GTA ACA GGC TTC CCG AGC TTA ATA Tyr Arg Met Tyr Lys Asn Asn Lys Val Thr Gly Phe Pro Ser Leu Ile 325 330 335	1302
ACC ATG TTC AGT GCG CCA AAC TAC CTG GAC ACA TAT CAT AAT AAA GCT Thr Met Phe Ser Ala Pro Asn Tyr Leu Asp Thr Tyr His Asn Lys Ala 340 345 350 355	1350
GCT GTG TTA AAA TAT GAA GAA AAC GTC ATG AAC ATC AGG CAG TTT CAC Ala Val Leu Lys Tyr Glu Glu Asn Val Met Asn Ile Arg Gln Phe His 360 365 370	1398
ATG TCT CCG CAC CCT TAC TGG TTG CCT GAT TTT ATG GAT GTT TTC ACC Met Ser Pro His Pro Tyr Trp Leu Pro Asp Phe Met Asp Val Phe Thr 375 380 385	1446
TGG TCA CTA CCT TTT GTT GGC GAA AAA GTT ACT AGC ATG TTA GTG TCT Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Ser Met Leu Val Ser 390 395 400	1494
ATA TTA AAC ATA TGT AGT GAG CAG GAA CTT GAC CCA GAA TCG GAA CCC Ile Leu Asn Ile Cys Ser Glu Gln Glu Leu Asp Pro Glu Ser Glu Pro 405 410 415	1542
AAA GCT GCG GAG GAG ACT GTA AAG GCA AGA GCA AAC GCA ACT AAG GAG Lys Ala Ala Glu Glu Thr Val Lys Ala Arg Ala Asn Ala Thr Lys Glu 420 425 430 435	1590
ACC GGC ACC CCA TCT GAT GAA AAG GCG TCA TCA GCG ATA TTA GAA GAT Thr Gly Thr Pro Ser Asp Glu Lys Ala Ser Ser Ala Ile Leu Glu Asp 440 445 450	1638
GAA ACC CGA AGA AAG GCT TTG AGA AAT AAG ATA TTA GCT ATT GCT AAA Glu Thr Arg Arg Lys Ala Leu Arg Asn Lys Ile Leu Ala Ile Ala Lys 455 460 465	1686
GTT TCA AGA ATG TTT TCG GTG CTA AGA GAA GAG AGC GAA AAA GTG GAA Val Ser Arg Met Phe Ser Val Leu Arg Glu Glu Ser Glu Lys Val Glu 470 475 480	1734
TAT TTG AAA ACT ATG AAT GCC GGT GTC TTA CCT CGT GGT GCT CTA GCT Tyr Leu Lys Thr Met Asn Ala Gly Val Leu Pro Arg Gly Ala Leu Ala 485 490 495	1782

54

CGT GGG ACT GAA GGT TTG AAT GAA ACG CTA AGC ACT TTT GAA AAG GCT	1830
Arg Gly Thr Glu Gly Leu Asn Glu Thr Leu Ser Thr Phe Glu Lys Ala	
500 505 510 515	
AGA AAG GAA GAC CTT ATT AAT GAA AAA TTA CCA CCA TCT TTA TCG GAG	1878
Arg Lys Glu Asp Leu Ile Asn Glu Lys Leu Pro Pro Ser Leu Ser Glu	
520 525 530	
GTT GAA CAA GAG AAG ATT AAA TAC TAC GAA AAA ATA TTA AAG GGA GCG	1926
Val Glu Gln Glu Lys Ile Lys Tyr Tyr Glu Lys Ile Leu Lys Gly Ala	
535 540 545	
GAG AAA AAG CCA CAA CTG TGATAAATCT TCATTTTATT	1964
Glu Lys Lys Pro Gln Leu	
550	

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 553 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met	Ser	Lys	Asp	Leu	Asn	Ser	Ser	Arg	Ile	Lys	Ile	Ile	Lys	Pro	Asn	
1				5					10					15		
Asp	Ser	Tyr	Ile	Lys	Val	Asp	Arg	Lys	Lys	Asp	Leu	Thr	Lys	Tyr	Glu	
			20					25					30			
Leu	Glu	Asn	Gly	Lys	Val	Ile	Ser	Thr	Lys	Asp	Arg	Ser	Tyr	Ala	Ser	
		35					40					45				
Val	Pro	Ala	Ile	Thr	Gly	Lys	Ile	Pro	Ser	Asp	Glu	Glu	Val	Phe	Asp	
	50					55					60					
Ser	Lys	Thr	Gly	Leu	Pro	Asn	His	Ser	Phe	Leu	Arg	Glu	His	Phe	Phe	
65				70					75					80		
His	Glu	Gly	Arg	Leu	Ser	Lys	Glu	Gln	Ala	Ile	Lys	Ile	Leu	Asn	Met	
			85					90						95		
Ser	Thr	Val	Ala	Leu	Ser	Lys	Glu	Pro	Asn	Leu	Leu	Lys	Leu	Lys	Ala	
		100						105					110			
Pro	Ile	Thr	Ile	Cys	Gly	Asp	Ile	His	Gly	Gln	Tyr	Tyr	Asp	Leu	Leu	
	115					120						125				
Lys	Leu	Phe	Glu	Val	Gly	Gly	Asp	Pro	Ala	Glu	Ile	Asp	Tyr	Leu	Phe	
	130					135					140					
Leu	Gly	Asp	Tyr	Val	Asp	Arg	Gly	Ala	Phe	Ser	Phe	Glu	Cys	Leu	Ile	
145				150					155					160		
Tyr	Leu	Tyr	Ser	Leu	Lys	Leu	Asn	Asn	Leu	Gly	Arg	Phe	Trp	Met	Leu	
			165					170						175		
Arg	Gly	Asn	His	Glu	Cys	Lys	His	Leu	Thr	Ser	Tyr	Phe	Thr	Phe	Lys	
		180						185					190			
Asn	Glu	Met	Leu	His	Lys	Tyr	Asp	Met	Glu	Val	Tyr	Asp	Ala	Cys	Cys	
	195					200						205				



55

Arg Ser Phe Asn Val Leu Pro Leu Ala Ala Leu Met Asn Gly Gln Tyr  
 210 215 220  
 Phe Cys Val His Gly Gly Ile Ser Pro Glu Leu Lys Ser Val Glu Asp  
 225 230 235 240  
 Val Asn Lys Ile Asn Arg Phe Arg Glu Ile Pro Ser Arg Gly Leu Met  
 245 250 255  
 Cys Asp Leu Leu Trp Ala Asp Pro Val Glu Asn Tyr Asp Asp Ala Arg  
 260 265 270  
 Asp Gly Ser Glu Phe Asp Gln Ser Glu Asp Glu Phe Val Pro Asn Ser  
 275 280 285  
 Leu Arg Gly Cys Ser Phe Ala Phe Thr Phe Lys Ala Ser Cys Lys Phe  
 290 295 300  
 Leu Lys Ala Asn Gly Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln  
 305 310 315 320  
 Asp Ala Gly Tyr Arg Met Tyr Lys Asn Asn Lys Val Thr Gly Phe Pro  
 325 330 335  
 Ser Leu Ile Thr Met Phe Ser Ala Pro Asn Tyr Leu Asp Thr Tyr His  
 340 345 350  
 Asn Lys Ala Ala Val Leu Lys Tyr Glu Glu Asn Val Met Asn Ile Arg  
 355 360 365  
 Gln Phe His Met Ser Pro His Pro Tyr Trp Leu Pro Asp Phe Met Asp  
 370 375 380  
 Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Ser Met  
 385 390 395 400  
 Leu Val Ser Ile Leu Asn Ile Cys Ser Glu Gln Glu Leu Asp Pro Glu  
 405 410 415  
 Ser Glu Pro Lys Ala Ala Glu Glu Thr Val Lys Ala Arg Ala Asn Ala  
 420 425 430  
 Thr Lys Glu Thr Gly Thr Pro Ser Asp Glu Lys Ala Ser Ser Ala Ile  
 435 440 445  
 Leu Glu Asp Glu Thr Arg Arg Lys Ala Leu Arg Asn Lys Ile Leu Ala  
 450 455 460  
 Ile Ala Lys Val Ser Arg Met Phe Ser Val Leu Arg Glu Glu Ser Glu  
 465 470 475 480  
 Lys Val Glu Tyr Leu Lys Thr Met Asn Ala Gly Val Leu Pro Arg Gly  
 485 490 495  
 Ala Leu Ala Arg Gly Thr Glu Gly Leu Asn Glu Thr Leu Ser Thr Phe  
 500 505 510  
 Glu Lys Ala Arg Lys Glu Asp Leu Ile Asn Glu Lys Leu Pro Pro Ser  
 515 520 525  
 Leu Ser Glu Val Glu Gln Glu Lys Ile Lys Tyr Tyr Glu Lys Ile Leu  
 530 535 540  
 Lys Gly Ala Glu Lys Lys Pro Gln Leu  
 545 550

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2353 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: genomic DNA fragment containing full  
 CNA2 coding sequence

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 262..2073

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATAGTCTATA ATACGTTTGA TACAGCTAGA TATCGCTAGC GCCAACATTG TCCCCCTCTC	60
TTGATCAATG CTTTTTTTCG GCCCGAGACA AATGAGAAAA TGTCCTAAAA ATACCTTTCA	120
TCAAGACTCC TATTTTTCCT TAGAAAAAAC ATATATCCAA CTGGAACAGT ATTAAGCCAA	180
TTGCTACGAT ACAAACAAAA GGAGATATTC CTTCCCTCCC ATAGAGTCAC ACAGGAGCCA	240
GTACTTCTTC TTGAACCCGC A ATG TCT TCA GAC GCT ATA AGA AAT ACT GAG	291
Met Ser Ser Asp Ala Ile Arg Asn Thr Glu	
1 5 10	
CAG ATA AAC GCC GCT ATT AAA ATT ATA GAA AAC AAA ACA GAG CGT CCG	339
Gln Ile Asn Ala Ala Ile Lys Ile Ile Glu Asn Lys Thr Glu Arg Pro	
15 20 25	
CAA TCG TCC ACA ACC CCT ATA GAT TCG AAG GCT AGT ACA GTT GCT GCT	387
Gln Ser Ser Thr Thr Pro Ile Asp Ser Lys Ala Ser Thr Val Ala Ala	
30 35 40	
GCT AAT TCC ACG GCC ACA GAA ACT TCC AGA GAC CTT ACA CAA TAT ACC	435
Ala Asn Ser Thr Ala Thr Glu Thr Ser Arg Asp Leu Thr Gln Tyr Thr	
45 50 55	
CTA GAT GAC GGA AGA GTC GTA TCG ACA AAC CGC AGA ATA ATG AAT AAA	483
Leu Asp Asp Gly Arg Val Val Ser Thr Asn Arg Arg Ile Met Asn Lys	
60 65 70	
GTG CCC GCC ATC ACG TCA CAT GTT CCT ACA GAT GAA GAG CTG TTC CAG	531
Val Pro Ala Ile Thr Ser His Val Pro Thr Asp Glu Glu Leu Phe Gln	
75 80 85 90	
CCC AAT GGG ATA CCT CGT CAC GAA TTC CTA AGA GAT CAT TTC AAG CGC	579
Pro Asn Gly Ile Pro Arg His Glu Phe Leu Arg Asp His Phe Lys Arg	
95 100 105	
GAG GGC AAA TTG TCG GCT GCG CAG GCG GCC AGG ATC GTT ACA CTT GCA	627
Glu Gly Lys Leu Ser Ala Ala Gln Ala Ala Arg Ile Val Thr Leu Ala	
110 115 120	
ACG GAA CTC TTC AGC AAA GAA CCC AAC CTT ATA TCT GTT CCC GCC CCA	675
Thr Glu Leu Phe Ser Lys Glu Pro Asn Leu Ile Ser Val Pro Ala Pro	
125 130 135	

57

ATC ACA GTT TGC GGT GAT ATC CAT GGC CAG TAC TTT GAC CTT TTG AAG Ile Thr Val Cys Gly Asp Ile His Gly Gln Tyr Phe Asp Leu Leu Lys 140 145 150	723
CTA TTC GAA GTT GGC GGA GAT CCG GCC ACT ACA TCG TAT TTG TTC TTG Leu Phe Glu Val Gly Gly Asp Pro Ala Thr Thr Ser Tyr Leu Phe Leu 155 160 165 170	771
GGA GAC TAT GTC GAC AGA GGG TCC TTT TCG TTT GAG TGT CTT ATT TAT Gly Asp Tyr Val Asp Arg Gly Ser Phe Ser Phe Glu Cys Leu Ile Tyr 175 180 185	819
TTA TAT TCT TTG AAG CTG AAT TTT AAC GAC CAT TTC TGG CTA CTG AGG Leu Tyr Ser Leu Lys Leu Asn Phe Asn Asp His Phe Trp Leu Leu Arg 190 195 200	867
GGT AAC CAC GAA TGT AAG CAT CTA ACG TCA TAT TTC ACT TTC AAA AAT Gly Asn His Glu Cys Lys His Leu Thr Ser Tyr Phe Thr Phe Lys Asn 205 210 215	915
GAA ATG CTG CAC AAG TAC AAT CTA GAT ATT TAC GAG AAA TGC TGC GAA Glu Met Leu His Lys Tyr Asn Leu Asp Ile Tyr Glu Lys Cys Cys Glu 220 225 230	963
TCG TTT AAC AAC TTG CCC CTG GCT GCG TTA ATG AAC GGA CAG TAT CTT Ser Phe Asn Asn Leu Pro Leu Ala Ala Leu Met Asn Gly Gln Tyr Leu 235 240 245 250	1011
TGT GTT CAT GGT GGT ATA TCT CCC GAG TTA AAC TCT TTA CAG GAC ATT Cys Val His Gly Gly Ile Ser Pro Glu Leu Asn Ser Leu Gln Asp Ile 255 260 265	1059
AAC AAC CTA AAT AGA TTC AGG GAG ATT CCC TCT CAT GGC CTG ATG TGT Asn Asn Leu Asn Arg Phe Arg Glu Ile Pro Ser His Gly Leu Met Cys 270 275 280	1107
GAT CTG TTG TGG GCT GAC CCG ATT GAA GAG TAC GAC GAA GTC TTG GAT Asp Leu Leu Trp Ala Asp Pro Ile Glu Glu Tyr Asp Glu Val Leu Asp 285 290 295	1155
AAA GAC TTG ACT GAG GAA GAC ATA GTG AAC TCC AAA ACC ATG GTT CCT Lys Asp Leu Thr Glu Glu Asp Ile Val Asn Ser Lys Thr Met Val Pro 300 305 310	1203
CAT CAT GGC AAG ATG GCA CCT TCA AGG GAT ATG TTT GTC CCA AAC TCA His His Gly Lys Met Ala Pro Ser Arg Asp Met Phe Val Pro Asn Ser 315 320 325 330	1251
GTA AGG GGC TGT TCA TAT GCC TTC ACG TAT CGT GCT GCG TGC CAT TTT Val Arg Gly Cys Ser Tyr Ala Phe Thr Tyr Arg Ala Ala Cys His Phe 335 340 345	1299
CTG CAA GAG ACT GGC CTG TTG TCC ATC ATC AGG GCA CAC GAG GCT CAA Leu Gln Glu Thr Gly Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln 350 355 360	1347
GAC GCT GGT TAT AGA ATG TAC AAA AAT ACC AAG ACT TTG GGC TTT CCC Asp Ala Gly Tyr Arg Met Tyr Lys Asn Thr Lys Thr Leu Gly Phe Pro 365 370 375	1395
TCT CTT TTG ACC CTT TTC AGT GCG CCT AAC TAC TTG GAC ACC TAC AAT Ser Leu Leu Thr Leu Phe Ser Ala Pro Asn Tyr Leu Asp Thr Tyr Asn 380 385 390	1443
AAT AAG GCT GCC ATA TTG AAA TAC GAA AAT AAT GTT ATG AAT ATC AGA Asn Lys Ala Ala Ile Leu Lys Tyr Glu Asn Asn Val Met Asn Ile Arg 395 400 405 410	1491

58

CAA TTC AAC ATG ACT CCA CAC CCC TAT TGG TTA CCA GAT TTC ATG GAC Gln Phe Asn Met Thr Pro His Pro Tyr Trp Leu Pro Asp Phe Met Asp 415 420 425	1539
GTT TTC ACG TGG TCC TTG CCA TTT GTT GGT GAA AAA GTT ACA GAG ATG Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu Met 430 435 440	1587
CTT GTC GCA ATT CTA AAC ATC TGT ACT GAA GAT GAG CTG GAA AAC GAC Leu Val Ala Ile Leu Asn Ile Cys Thr Glu Asp Glu Leu Glu Asn Asp 445 450 455	1635
ACC CCC GTC ATT GAA GAA TTA GTT GGT ACC GAT AAA AAA TTG CCA CAA Thr Pro Val Ile Glu Glu Leu Val Gly Thr Asp Lys Lys Leu Pro Gln 460 465 470	1683
GCT GGT AAG TCG GAA GCA ACT CCA CAA CCA GCC ACT TCG GCG TCG CCT Ala Gly Lys Ser Glu Ala Thr Pro Gln Pro Ala Thr Ser Ala Ser Pro 475 480 485 490	1731
AAA CAT GCT TCC ATT TTA GAT GAC GAA CAT CGA AGG AAA GCC TTA CGA Lys His Ala Ser Ile Leu Asp Asp Glu His Arg Arg Lys Ala Leu Arg 495 500 505	1779
AAT AAG ATT CTG GCC GTC GCC AAA GTT TCC AGA ATG TAT TCT GTT CTC Asn Lys Ile Leu Ala Val Ala Lys Val Ser Arg Met Tyr Ser Val Leu 510 515 520	1827
AGA GAA GAA ACC AAT AAA GTT CAG TTT TTA AAA GAT CAC AAT TCA GGC Arg Glu Glu Thr Asn Lys Val Gln Phe Leu Lys Asp His Asn Ser Gly 525 530 535	1875
GTG TTG CCA CGT GGC GCT TTA TCT AAT GGT GTA AAG GGT TTA GAT GAA Val Leu Pro Arg Gly Ala Leu Ser Asn Gly Val Lys Gly Leu Asp Glu 540 545 550	1923
GCC CTG TCT ACC TTT GAA AGG GCA AGA AAG CAC GAT TTA ATT AAT GAA Ala Leu Ser Thr Phe Glu Arg Ala Arg Lys His Asp Leu Ile Asn Glu 555 560 565 570	1971
AAA TTA CCG CCT TCA CTA GAC GAA CTG AAA AAC GAA AAT AAG AAG TAC Lys Leu Pro Pro Ser Leu Asp Glu Leu Lys Asn Glu Asn Lys Lys Tyr 575 580 585	2019
TAC GAA AAA GTT TGG CAG AAA GTA CAT GAA CAT GAT GCA AAG AAT GAT Tyr Glu Lys Val Trp Gln Lys Val His Glu His Asp Ala Lys Asn Asp 590 595 600	2067
AGC AAA TAGAGAAAGC TCCTATTTCC ACTGTACATA CTTCAATAAG TAAGTAAGTT Ser Lys	2123
GCATTAATTA TCTATTTAGA AGCTAGATGC TCCTCAAATG CACAGAATCA TATAGCGTTT	2183
TATTAGGTCT GTTCTTTATT TTAGTTTTGT TGATCTCTAT GAAGGTATAT TTATATGCAA	2243
AAATAAACTT TTAAATATCT ATGGATGCTT ACTCAATTGT ATAGACGTTT TTCATAGGAG	2303
TGCAAATTAT GGACACCACC TTCTAATTGA GCAGAAGCGG TTCTGAATTC	2353

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 604 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ser Ser Asp Ala Ile Arg Asn Thr Glu Gln Ile Asn Ala Ala Ile  
 1 5 10 15  
 Lys Ile Ile Glu Asn Lys Thr Glu Arg Pro Gln Ser Ser Thr Thr Pro  
 20 25 30  
 Ile Asp Ser Lys Ala Ser Thr Val Ala Ala Ala Asn Ser Thr Ala Thr  
 35 40 45  
 Glu Thr Ser Arg Asp Leu Thr Gln Tyr Thr Leu Asp Asp Gly Arg Val  
 50 55 60  
 Val Ser Thr Asn Arg Arg Ile Met Asn Lys Val Pro Ala Ile Thr Ser  
 65 70 75 80  
 His Val Pro Thr Asp Glu Glu Leu Phe Gln Pro Asn Gly Ile Pro Arg  
 85 90 95  
 His Glu Phe Leu Arg Asp His Phe Lys Arg Glu Gly Lys Leu Ser Ala  
 100 105 110  
 Ala Gln Ala Ala Arg Ile Val Thr Leu Ala Thr Glu Leu Phe Ser Lys  
 115 120 125  
 Glu Pro Asn Leu Ile Ser Val Pro Ala Pro Ile Thr Val Cys Gly Asp  
 130 135 140  
 Ile His Gly Gln Tyr Phe Asp Leu Leu Lys Leu Phe Glu Val Gly Gly  
 145 150 155 160  
 Asp Pro Ala Thr Thr Ser Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg  
 165 170 175  
 Gly Ser Phe Ser Phe Glu Cys Leu Ile Tyr Leu Tyr Ser Leu Lys Leu  
 180 185 190  
 Asn Phe Asn Asp His Phe Trp Leu Leu Arg Gly Asn His Glu Cys Lys  
 195 200 205  
 His Leu Thr Ser Tyr Phe Thr Phe Lys Asn Glu Met Leu His Lys Tyr  
 210 215 220  
 Asn Leu Asp Ile Tyr Glu Lys Cys Cys Glu Ser Phe Asn Asn Leu Pro  
 225 230 235 240  
 Leu Ala Ala Leu Met Asn Gly Gln Tyr Leu Cys Val His Gly Gly Ile  
 245 250 255  
 Ser Pro Glu Leu Asn Ser Leu Gln Asp Ile Asn Asn Leu Asn Arg Phe  
 260 265 270  
 Arg Glu Ile Pro Ser His Gly Leu Met Cys Asp Leu Leu Trp Ala Asp  
 275 280 285  
 Pro Ile Glu Glu Tyr Asp Glu Val Leu Asp Lys Asp Leu Thr Glu Glu  
 290 295 300  
 Asp Ile Val Asn Ser Lys Thr Met Val Pro His His Gly Lys Met Ala  
 305 310 315 320  
 Pro Ser Arg Asp Met Phe Val Pro Asn Ser Val Arg Gly Cys Ser Tyr  
 325 330 335

60

Ala Phe Thr Tyr Arg Ala Ala Cys His Phe Leu Gln Glu Thr Gly Leu  
                             340                            345                            350  
 Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met  
                             355                            360                            365  
 Tyr Lys Asn Thr Lys Thr Leu Gly Phe Pro Ser Leu Leu Thr Leu Phe  
                             370                            375                            380  
 Ser Ala Pro Asn Tyr Leu Asp Thr Tyr Asn Asn Lys Ala Ala Ile Leu  
                             385                            390                            395                            400  
 Lys Tyr Glu Asn Asn Val Met Asn Ile Arg Gln Phe Asn Met Thr Pro  
                             405                            410                            415  
 His Pro Tyr Trp Leu Pro Asp Phe Met Asp Val Phe Thr Trp Ser Leu  
                             420                            425                            430  
 Pro Phe Val Gly Glu Lys Val Thr Glu Met Leu Val Ala Ile Leu Asn  
                             435                            440                            445  
 Ile Cys Thr Glu Asp Glu Leu Glu Asn Asp Thr Pro Val Ile Glu Glu  
                             450                            455                            460  
 Leu Val Gly Thr Asp Lys Lys Leu Pro Gln Ala Gly Lys Ser Glu Ala  
                             465                            470                            475                            480  
 Thr Pro Gln Pro Ala Thr Ser Ala Ser Pro Lys His Ala Ser Ile Leu  
                             485                            490                            495  
 Asp Asp Glu His Arg Arg Lys Ala Leu Arg Asn Lys Ile Leu Ala Val  
                             500                            505                            510  
 Ala Lys Val Ser Arg Met Tyr Ser Val Leu Arg Glu Glu Thr Asn Lys  
                             515                            520                            525  
 Val Gln Phe Leu Lys Asp His Asn Ser Gly Val Leu Pro Arg Gly Ala  
                             530                            535                            540  
 Leu Ser Asn Gly Val Lys Gly Leu Asp Glu Ala Leu Ser Thr Phe Glu  
                             545                            550                            555                            560  
 Arg Ala Arg Lys His Asp Leu Ile Asn Glu Lys Leu Pro Pro Ser Leu  
                             565                            570                            575  
 Asp Glu Leu Lys Asn Glu Asn Lys Lys Tyr Tyr Glu Lys Val Trp Gln  
                             580                            585                            590  
 Lys Val His Glu His Asp Ala Lys Asn Asp Ser Lys  
                             595                            600

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 812 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: genomic DNA fragment containing full

## CNB1 coding sequence

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 54..104

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 181..652

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACTTGGTAAC TCAATGGTGA TCAGAATCCA TAGAAGCATT TTTATTCTT AAA ATG	56
Met	
1	
GGT GCT GCT CCT TCC AAA ATT GTG GAT GGT CTT TTA GAA GAT ACA AAT	104
Gly Ala Ala Pro Ser Lys Ile Val Asp Gly Leu Leu Glu Asp Thr Asn	
5 10 15	
TGTATGTACA CTTCGGAGTG AGGAAAAGAA AGAAAGGGGA AATTAACCGA TTTTACTAAC	164
ACTGACACTT TGAACA GTT GAT AGA GAT GAA ATT GAA AGG TTA AGG AAG	213
Val Asp Arg Asp Glu Ile Glu Arg Leu Arg Lys	
1 5 10	
AGA TTC ATG AAA TTA GAT AGA GAT AGC TCA GGG TCT ATT GAT AAA AAT	261
Arg Phe Met Lys Leu Asp Arg Asp Ser Ser Gly Ser Ile Asp Lys Asn	
15 20 25	
GAA TTT ATG AGC ATT CCT GGC GTT TCG TCA AAC CCT CTT GCT GGA CGT	309
Glu Phe Met Ser Ile Pro Gly Val Ser Ser Asn Pro Leu Ala Gly Arg	
30 35 40	
ATA ATG GAG GTT TTC GAT GCT GAT AAT AGT GGG GAC GTG GAT TTT CAA	357
Ile Met Glu Val Phe Asp Ala Asp Asn Ser Gly Asp Val Asp Phe Gln	
45 50 55	
GAG TTC ATC ACA GGA TTA TCC ATT TTC AGT GGG CGT GGG TCC AAG GAC	405
Glu Phe Ile Thr Gly Leu Ser Ile Phe Ser Gly Arg Gly Ser Lys Asp	
60 65 70 75	
GAA AAG TTA AGA TTC GCC TTC AAA ATC TAC GAC ATT GAC AAG GAC GGT	453
Glu Lys Leu Arg Phe Ala Phe Lys Ile Tyr Asp Ile Asp Lys Asp Gly	
80 85 90	
TTC ATA TCC AAT GGT GAG TTG TTC ATC GTG TTG AAG ATT ATG GTA GGT	501
Phe Ile Ser Asn Gly Glu Leu Phe Ile Val Leu Lys Ile Met Val Gly	
95 100 105	
TCT AAT CTG GAC GAT GAA CAG CTG CAA CAG ATA GTA GAT AGG ACG ATA	549
Ser Asn Leu Asp Asp Glu Gln Leu Gln Gln Ile Val Asp Arg Thr Ile	
110 115 120	
GTG GAA AAC GAT AGC GAC GGC GAC GGA CGT TTA AGT TTC GAG GAG TTT	597
Val Glu Asn Asp Ser Asp Gly Asp Gly Arg Leu Ser Phe Glu Glu Phe	
125 130 135	
AAG AAT GCT ATC GAA ACC ACA GAA GTG GCC AAG AGT CTG ACA TTG CAA	645
Lys Asn Ala Ile Glu Thr Thr Glu Val Ala Lys Ser Leu Thr Leu Gln	
140 145 150 155	
TAC GATGTGTAAG ACTAGGGGAC ACTTCATTCA TTTATGGTAT GCCAATATTT	698
Tyr Asp	

62

TTAAGAAAAG AAGAATAATA CGCGATATTG TTTTSTAAGG AAGGAACGCA CACTCGCCCA 758  
 GTTAGAGTGC TGATGATATA TACATATATA TATGTATATG TAACAAACAA TAAG 812

## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 17 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Gly Ala Ala Pro Ser Lys Ile Val Asp Gly Leu Leu Glu Asp Thr  
 1 5 10 15  
 Asn

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Val Asp Arg Asp Glu Ile Glu Arg Leu Arg Lys Arg Phe Met Lys Leu  
 1 5 10 15  
 Asp Arg Asp Ser Ser Gly Ser Ile Asp Lys Asn Glu Phe Met Ser Ile  
 20 25 30  
 Pro Gly Val Ser Ser Asn Pro Leu Ala Gly Arg Ile Met Glu Val Phe  
 35 40 45  
 Asp Ala Asp Asn Ser Gly Asp Val Asp Phe Gln Glu Phe Ile Thr Gly  
 50 55 60  
 Leu Ser Ile Phe Ser Gly Arg Gly Ser Lys Asp Glu Lys Leu Arg Phe  
 65 70 75 80  
 Ala Phe Lys Ile Tyr Asp Ile Asp Lys Asp Gly Phe Ile Ser Asn Gly  
 85 90 95  
 Glu Leu Phe Ile Val Leu Lys Ile Met Val Gly Ser Asn Leu Asp Asp  
 100 105 110  
 Glu Gln Leu Gln Gln Ile Val Asp Arg Thr Ile Val Glu Asn Asp Ser  
 115 120 125  
 Asp Gly Asp Gly Arg Leu Ser Phe Glu Glu Phe Lys Asn Ala Ile Glu  
 130 135 140  
 Thr Thr Glu Val Ala Lys Ser Leu Thr Leu Gln Tyr Asp  
 145 150 155



## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 524 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: coding sequence of CNB1

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..524

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATG GGT GCT GCT CCT TCC AAA ATT GTG GAT GGT CTT TTA GAA GAT ACA	48
Met Gly Ala Ala Pro Ser Lys Ile Val Asp Gly Leu Leu Glu Asp Thr	
1 5 10 15	
AAT TTT GAT AGA GAT GAA ATT GAA AGG TTA AGG AAG AGA TTC ATG AAA	96
Asn Phe Asp Arg Asp Glu Ile Glu Arg Leu Arg Lys Arg Phe Met Lys	
20 25 30	
TTA GAT AGA GAT AGC TCA GGG TCT ATT GAT AAA AAT GAA TTT ATG AGC	144
Leu Asp Arg Asp Ser Ser Gly Ser Ile Asp Lys Asn Glu Phe Met Ser	
35 40 45	
ATT CCT GGC GTT TCG TCA AAC CCT CTT GCT GGA CGT ATA ATG GAG GTT	192
Ile Pro Gly Val Ser Ser Asn Pro Leu Ala Gly Arg Ile Met Glu Val	
50 55 60	
TTC GAT GCT GAT AAT AGT GGG GAC GTG GAT TTT CAA GAG TTC ATC ACA	240
Phe Asp Ala Asp Asn Ser Gly Asp Val Asp Phe Gln Glu Phe Ile Thr	
65 70 75 80	
GGA TTA TCC ATT TTC AGT GGG CGT GGG TCC AAG GAC GAA AAG TTA AGA	288
Gly Leu Ser Ile Phe Ser Gly Arg Gly Ser Lys Asp Glu Lys Leu Arg	
85 90 95	
TTC GCC TTC AAA ATC TAC GAC ATT GAC AAG GAC GGT TTC ATA TCC AAT	336
Phe Ala Phe Lys Ile Tyr Asp Ile Asp Lys Asp Gly Phe Ile Ser Asn	
100 105 110	
GGT GAG TTG TTC ATC GTG TTG AAG ATT ATG GTA GGT TCT AAT CTG GAC	384
Gly Glu Leu Phe Ile Val Leu Lys Ile Met Val Gly Ser Asn Leu Asp	
115 120 125	
GAT GAA CAG CTG CAA CAG ATA GTA GAT AGG ACG ATA GTG GAA AAC GAT	432
Asp Glu Gln Leu Gln Gln Ile Val Asp Arg Thr Ile Val Glu Asn Asp	
130 135 140	
AGC GAC GGC GAC GGA CGT TTA AGT TTC GAG GAG TTT AAG AAT GCT ATC	480
Ser Asp Gly Asp Gly Arg Leu Ser Phe Glu Glu Phe Lys Asn Ala Ile	
145 150 155 160	
GAA ACC ACA GAA GTG GCC AAG AGT CTG ACA TTG CAA TAC GAT GT	524
Glu Thr Thr Glu Val Ala Lys Ser Leu Thr Leu Gln Tyr Asp	
165 170	

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## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 174 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

Met Gly Ala Ala Pro Ser Lys Ile Val Asp Gly Leu Leu Glu Asp Thr
 1             5             10             15
Asn Phe Asp Arg Asp Glu Ile Glu Arg Leu Arg Lys Arg Phe Met Lys
      20             25             30
Leu Asp Arg Asp Ser Ser Gly Ser Ile Asp Lys Asn Glu Phe Met Ser
      35             40             45
Ile Pro Gly Val Ser Ser Asn Pro Leu Ala Gly Arg Ile Met Glu Val
      50             55             60
Phe Asp Ala Asp Asn Ser Gly Asp Val Asp Phe Gln Glu Phe Ile Thr
      65             70             75             80
Gly Leu Ser Ile Phe Ser Gly Arg Gly Ser Lys Asp Glu Lys Leu Arg
      85             90             95
Phe Ala Phe Lys Ile Tyr Asp Ile Asp Lys Asp Gly Phe Ile Ser Asn
      100            105            110
Gly Glu Leu Phe Ile Val Leu Lys Ile Met Val Gly Ser Asn Leu Asp
      115            120            125
Asp Glu Gln Leu Gln Gln Ile Val Asp Arg Thr Ile Val Glu Asn Asp
      130            135            140
Ser Asp Gly Asp Gly Arg Leu Ser Phe Glu Glu Phe Lys Asn Ala Ile
      145            150            155            160
Glu Thr Thr Glu Val Ala Lys Ser Leu Thr Leu Gln Tyr Asp
      165            170

```

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1812 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: DNA fragment containing CNAIdeltaC coding sequence

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 286..1812

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTTTGTGCA TTTTGATATT CATCTATATC TATTTCAAAA TTTTTCATGT CATCGCCTCT	60
TGAAACATGA ATTTTCCAAT TCTGAAAAAG AACGTACTAC TGGGAAACAA AAGGGAAAAA	120
TGTATAAATC CTTTAATGTT TTTGAATCAA GAGGCATTAT TATAAAAGAA CGAAGCAAAG	180
CCTTTAATAT TTGCTTTATT AAAGGTATTA TTCAAAGAAA AGTTTTTTTA GATTCTTTTT	240
TTTTTGACGT ATTAGCTCAG CTGCCATAAA ACACTCTCAA CGCCA ATG TCG AAA	294
Met Ser Lys	
1	
GAC TTG AAT TCT TCA CGC ATC AAA ATC ATT AAA CCT AAT GAC TCT TAC	342
Asp Leu Asn Ser Ser Arg Ile Lys Ile Ile Lys Pro Asn Asp Ser Tyr	
5 10 15	
ATA AAG GTT GAC CGG AAA AAA GAT TTA ACA AAA TAC GAA TTA GAA AAC	390
Ile Lys Val Asp Arg Lys Lys Asp Leu Thr Lys Tyr Glu Leu Glu Asn	
20 25 30 35	
GGT AAA GTA ATT TCT ACT AAG GAC CGA TCC TAC GCT TCT GTA CCT GCC	438
Gly Lys Val Ile Ser Thr Lys Asp Arg Ser Tyr Ala Ser Val Pro Ala	
40 45 50	
ATA ACA GGA AAG ATA CCA AGT GAT GAG GAA GTA TTC GAC TCC AAG ACG	486
Ile Thr Gly Lys Ile Pro Ser Asp Glu Glu Val Phe Asp Ser Lys Thr	
55 60 65	
GGA TTA CCT AAT CAT TCC TTT TTA AGA GAG CAT TTC TTT CAT GAG GGT	534
Gly Leu Pro Asn His Ser Phe Leu Arg Glu His Phe Phe His Glu Gly	
70 75 80	
CGA CTT TCT AAG GAA CAG GCC ATA AAA ATC TTA AAT ATG TCA ACT GTA	582
Arg Leu Ser Lys Glu Gln Ala Ile Lys Ile Leu Asn Met Ser Thr Val	
85 90 95	
GCA TTG AGT AAA GAA CCC AAT CTA CTA AAA CTC AAA GCG CCA ATT ACT	630
Ala Leu Ser Lys Glu Pro Asn Leu Leu Lys Leu Lys Ala Pro Ile Thr	
100 105 110 115	
ATA TGT GGT GAT ATT CAC GGG CAG TAT TAT GAT TTA TTG AAA CTG TTT	678
Ile Cys Gly Asp Ile His Gly Gln Tyr Tyr Asp Leu Leu Lys Leu Phe	
120 125 130	
GAA GTT GGC GGT GAC CCC GCC GAA ATC GAC TAT TTA TTC TTG GGG GAT	726
Glu Val Gly Gly Asp Pro Ala Glu Ile Asp Tyr Leu Phe Leu Gly Asp	
135 140 145	
TAT GTT GAT AGA GGT GCA TTC TCT TTT GAG TGT CTG ATT TAT TTG TAC	774
Tyr Val Asp Arg Gly Ala Phe Ser Phe Glu Cys Leu Ile Tyr Leu Tyr	
150 155 160	
TCC TTG AAG TTG AAT AAT TTA GGG AGA TTT TGG ATG CTA AGA GGT AAC	822
Ser Leu Lys Leu Asn Asn Leu Gly Arg Phe Trp Met Leu Arg Gly Asn	
165 170 175	
CAT GAG TGT AAG CAC TTG ACC TCT TAT TTT ACT TTT AAG AAT GAG ATG	870
His Glu Cys Lys His Leu Thr Ser Tyr Phe Thr Phe Lys Asn Glu Met	
180 185 190 195	
TTG CAC AAA TAC GAT ATG GAA GTT TAC GAT GCT TGC TGC AGA TCA TTC	918
Leu His Lys Tyr Asp Met Glu Val Tyr Asp Ala Cys Cys Arg Ser Phe	
200 205 210	

AAT GTC TTA CCA TTA GCA GCT TTA ATG AAC GGA CAA TAT TTT TGT GTG Asn Val Leu Pro Leu Ala Ala Leu Met Asn Gly Gln Tyr Phe Cys Val 215 220 225	966
CAT GGT GGT ATC TCT CCA GAG TTA AAA TCA GTA GAG GAT GTT AAT AAA His Gly Gly Ile Ser Pro Glu Leu Lys Ser Val Glu Asp Val Asn Lys 230 235 240	1014
ATT AAT AGA TTT CGA GAA ATC CCA TCT CGT GGT CTC ATG TGT GAC CTA Ile Asn Arg Phe Arg Glu Ile Pro Ser Arg Gly Leu Met Cys Asp Leu 245 250 255	1062
CTA TGG GCC GAT CCT GTC GAA AAT TAT GAT GAT GCA AGA GAT GGT AGC Leu Trp Ala Asp Pro Val Glu Asn Tyr Asp Asp Ala Arg Asp Gly Ser 260 265 270 275	1110
GAA TTT GAT CAG AGC GAG GAT GAA TTC GTA CCT AAC AGT TTG AGG GGT Glu Phe Asp Gln Ser Glu Asp Glu Phe Val Pro Asn Ser Leu Arg Gly 280 285 290	1158
TGC TCT TTC GCC TTC ACT TTT AAA GCA TCA TGC AAG TTT TTG AAG GCA Cys Ser Phe Ala Phe Thr Phe Lys Ala Ser Cys Lys Phe Leu Lys Ala 295 300 305	1206
AAT GGT TTG TTA TCT ATT ATT AGA GCA CAC GAA GCA CAG GAT GCT GGG Asn Gly Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp Ala Gly 310 315 320	1254
TAC AGA ATG TAT AAA AAC AAT AAA GTA ACA GGC TTC CCG AGC TTA ATA Tyr Arg Met Tyr Lys Asn Asn Lys Val Thr Gly Phe Pro Ser Leu Ile 325 330 335	1302
ACC ATG TTC AGT GCG CCA AAC TAC CTG GAC ACA TAT CAT AAT AAA GCT Thr Met Phe Ser Ala Pro Asn Tyr Leu Asp Thr Tyr His Asn Lys Ala 340 345 350 355	1350
GCT GTG TTA AAA TAT GAA GAA AAC GTC ATG AAC ATC AGG CAG TTT CAC Ala Val Leu Lys Tyr Glu Glu Asn Val Met Asn Ile Arg Gln Phe His 360 365 370	1398
ATG TCT CCG CAC CCT TAC TGG TTG CCT GAT TTT ATG GAT GTT TTC ACC Met Ser Pro His Pro Tyr Trp Leu Pro Asp Phe Met Asp Val Phe Thr 375 380 385	1446
TGG TCA CTA CCT TTT GTT GGC GAA AAA GTT ACT AGC ATG TTA GTG TCT Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Ser Met Leu Val Ser 390 395 400	1494
ATA TTA AAC ATA TGT AGT GAG CAG GAA CTT GAC CCA GAA TCG GAA CCC Ile Leu Asn Ile Cys Ser Glu Gln Glu Leu Asp Pro Glu Ser Glu Pro 405 410 415	1542
AAA GCT GCG GAG GAG ACT GTA AAG GCA AGA GCA AAC GCA ACT AAG GAG Lys Ala Ala Glu Glu Thr Val Lys Ala Arg Ala Asn Ala Thr Lys Glu 420 425 430 435	1590
ACC GGC ACC CCA TCT GAT GAA AAG GCG TCA TCA GCG ATA TTA GAA GAT Thr Gly Thr Pro Ser Asp Glu Lys Ala Ser Ser Ala Ile Leu Glu Asp 440 445 450	1638
GAA ACC CGA AGA AAG GCT TTG AGA AAT AAG ATA TTA GCT ATT GCT AAA Glu Thr Arg Arg Lys Ala Leu Arg Asn Lys Ile Leu Ala Ile Ala Lys 455 460 465	1686
GTT TCA AGA ATG TTT TCG GTG CTA AGA GAA GAG AGC GAA AAA GTG GAA Val Ser Arg Met Phe Ser Val Leu Arg Glu Glu Ser Glu Lys Val Glu 470 475 480	1734

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TAT TTG AAA ACT ATG AAT GCC GGT GTC TTA CCT CGT GGT GCT CTA GCT 1782  
 Tyr Leu Lys Thr Met Asn Ala Gly Val Leu Pro Arg Gly Ala Leu Ala  
 485 490 495

CGT GGG ACT GAA GGT TTG AAT GAA ACG CTA 1812  
 Arg Gly Thr Glu Gly Leu Asn Glu Thr Leu  
 500 505

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 509 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ser Lys Asp Leu Asn Ser Ser Arg Ile Lys Ile Ile Lys Pro Asn  
 1 5 10 15  
 Asp Ser Tyr Ile Lys Val Asp Arg Lys Lys Asp Leu Thr Lys Tyr Glu  
 20 25 30  
 Leu Glu Asn Gly Lys Val Ile Ser Thr Lys Asp Arg Ser Tyr Ala Ser  
 35 40 45  
 Val Pro Ala Ile Thr Gly Lys Ile Pro Ser Asp Glu Glu Val Phe Asp  
 50 55 60  
 Ser Lys Thr Gly Leu Pro Asn His Ser Phe Leu Arg Glu His Phe Phe  
 65 70 75 80  
 His Glu Gly Arg Leu Ser Lys Glu Gln Ala Ile Lys Ile Leu Asn Met  
 85 90 95  
 Ser Thr Val Ala Leu Ser Lys Glu Pro Asn Leu Leu Lys Leu Lys Ala  
 100 105 110  
 Pro Ile Thr Ile Cys Gly Asp Ile His Gly Gln Tyr Tyr Asp Leu Leu  
 115 120 125  
 Lys Leu Phe Glu Val Gly Gly Asp Pro Ala Glu Ile Asp Tyr Leu Phe  
 130 135 140  
 Leu Gly Asp Tyr Val Asp Arg Gly Ala Phe Ser Phe Glu Cys Leu Ile  
 145 150 155 160  
 Tyr Leu Tyr Ser Leu Lys Leu Asn Asn Leu Gly Arg Phe Trp Met Leu  
 165 170 175  
 Arg Gly Asn His Glu Cys Lys His Leu Thr Ser Tyr Phe Thr Phe Lys  
 180 185 190  
 Asn Glu Met Leu His Lys Tyr Asp Met Glu Val Tyr Asp Ala Cys Cys  
 195 200 205  
 Arg Ser Phe Asn Val Leu Pro Leu Ala Ala Leu Met Asn Gly Gln Tyr  
 210 215 220  
 Phe Cys Val His Gly Gly Ile Ser Pro Glu Leu Lys Ser Val Glu Asp  
 225 230 235 240  
 Val Asn Lys Ile Asn Arg Phe Arg Glu Ile Pro Ser Arg Gly Leu Met  
 245 250 255

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Cys Asp Leu Leu Trp Ala Asp Pro Val Glu Asn Tyr Asp Asp Ala Arg  
 260 265 270  
 Asp Gly Ser Glu Phe Asp Gln Ser Glu Asp Glu Phe Val Pro Asn Ser  
 275 280 285  
 Leu Arg Gly Cys Ser Phe Ala Phe Thr Phe Lys Ala Ser Cys Lys Phe  
 290 295 300  
 Leu Lys Ala Asn Gly Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln  
 305 310 315 320  
 Asp Ala Gly Tyr Arg Met Tyr Lys Asn Asn Lys Val Thr Gly Phe Pro  
 325 330 335  
 Ser Leu Ile Thr Met Phe Ser Ala Pro Asn Tyr Leu Asp Thr Tyr His  
 340 345 350  
 Asn Lys Ala Ala Val Leu Lys Tyr Glu Glu Asn Val Met Asn Ile Arg  
 355 360 365  
 Gln Phe His Met Ser Pro His Pro Tyr Trp Leu Pro Asp Phe Met Asp  
 370 375 380  
 Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Ser Met  
 385 390 395 400  
 Leu Val Ser Ile Leu Asn Ile Cys Ser Glu Gln Glu Leu Asp Pro Glu  
 405 410 415  
 Ser Glu Pro Lys Ala Ala Glu Glu Thr Val Lys Ala Arg Ala Asn Ala  
 420 425 430  
 Thr Lys Glu Thr Gly Thr Pro Ser Asp Glu Lys Ala Ser Ser Ala Ile  
 435 440 445  
 Leu Glu Asp Glu Thr Arg Arg Lys Ala Leu Arg Asn Lys Ile Leu Ala  
 450 455 460  
 Ile Ala Lys Val Ser Arg Met Phe Ser Val Leu Arg Glu Glu Ser Glu  
 465 470 475 480  
 Lys Val Glu Tyr Leu Lys Thr Met Asn Ala Gly Val Leu Pro Arg Gly  
 485 490 495  
 Ala Leu Ala Arg Gly Thr Glu Gly Leu Asn Glu Thr Leu  
 500 505

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1767 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: DNA fragment containing CNA2deltaC coding sequence

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 262..1767

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATAGTCTATA ATACGTTTGA TACAGCTAGA TATCGCTAGC GCCAACATTG TCCCCCTCTC	60
TTGATCAATG CTTTTTTTCG GCCCGAGACA AATGAGAAAA TGTCCTAAAA ATACCTTTCA	120
TCAAGACTCC TATTTTTCCT TAGAAAAAAC ATATATCCAA CTGGAACAGT ATTAAGCCAA	180
TTGCTACGAT ACAAACAAAA GGAGATATTC CTTCCTCCC ATAGAGTCAC ACAGGAGCCA	240
GTACTTCTTC TTGAACCCGC A ATG TCT TCA GAC GCT ATA AGA AAT ACT GAG	291
Met Ser Ser Asp Ala Ile Arg Asn Thr Glu	
1 5 1 10	
CAG ATA AAC GCC GCT ATT AAA ATT ATA GAA AAC AAA ACA GAG CGT CCG	339
Gln Ile Asn Ala Ala Ile Lys Ile Ile Glu Asn Lys Thr Glu Arg Pro	
15 20 25	
CAA TCG TCC ACA ACC CCT ATA GAT TCG AAG GCT AGT ACA GTT GCT GCT	387
Gln Ser Ser Thr Thr Pro Ile Asp Ser Lys Ala Ser Thr Val Ala Ala	
30 35 40	
GCT AAT TCC ACG GCC ACA GAA ACT TCC AGA GAC CTT ACA CAA TAT ACC	435
Ala Asn Ser Thr Ala Thr Glu Thr Ser Arg Asp Leu Thr Gln Tyr Thr	
45 50 55	
CTA GAT GAC GGA AGA GTC GTA TCG ACA AAC CGC AGA ATA ATG AAT AAA	483
Leu Asp Asp Gly Arg Val Val Ser Thr Asn Arg Arg Ile Met Asn Lys	
60 65 70	
GTG CCC GCC ATC ACG TCA CAT GTT CCT ACA GAT GAA GAG CTG TTC CAG	531
Val Pro Ala Ile Thr Ser His Val Pro Thr Asp Glu Glu Leu Phe Gln	
75 80 85 90	
CCC AAT GGG ATA CCT CGT CAC GAA TTC CTA AGA GAT CAT TTC AAG CGC	579
Pro Asn Gly Ile Pro Arg His Glu Phe Leu Arg Asp His Phe Lys Arg	
95 100 105	
GAG GGC AAA TTG TCG GCT GCG CAG GCG GCC AGG ATC GTT ACA CTT GCA	627
Glu Gly Lys Leu Ser Ala Ala Gln Ala Ala Arg Ile Val Thr Leu Ala	
110 115 120	
ACG GAA CTC TTC AGC AAA GAA CCC AAC CTT ATA TCT GTT CCC GCC CCA	675
Thr Glu Leu Phe Ser Lys Glu Pro Asn Leu Ile Ser Val Pro Ala Pro	
125 130 135	
ATC ACA GTT TGC GGT GAT ATC CAT GGC CAG TAC TTT GAC CTT TTG AAG	723
Ile Thr Val Cys Gly Asp Ile His Gly Gln Tyr Phe Asp Leu Leu Lys	
140 145 150	
CTA TTC GAA GTT GGC GGA GAT CCG GCC ACT ACA TCG TAT TTG TTC TTG	771
Leu Phe Glu Val Gly Gly Asp Pro Ala Thr Thr Ser Tyr Leu Phe Leu	
155 160 165 170	
GGA GAC TAT GTC GAC AGA GGG TCC TTT TCG TTT GAG TGT CTT ATT TAT	819
Gly Asp Tyr Val Asp Arg Gly Ser Phe Ser Phe Glu Cys Leu Ile Tyr	
175 180 185	
TTA TAT TCT TTG AAG CTG AAT TTT AAC GAC CAT TTC TGG CTA CTG AGG	867
Leu Tyr Ser Leu Lys Leu Asn Phe Asn Asp His Phe Trp Leu Leu Arg	
190 195 200	

GGT AAC CAC GAA TGT AAG CAT CTA ACG TCA TAT TTC ACT TTC AAA AAT Gly Asn His Glu Cys Lys His Leu Thr Ser Tyr Phe Thr Phe Lys Asn 205 210 215	915
GAA ATG CTG CAC AAG TAC AAT CTA GAT ATT TAC GAG AAA TGC TGC GAA Glu Met Leu His Lys Tyr Asn Leu Asp Ile Tyr Glu Lys Cys Cys Glu 220 225 230	963
TCG TTT AAC AAC TTG CCC CTG GCT GCG TTA ATG AAC GGA CAG TAT CTT Ser Phe Asn Asn Leu Pro Leu Ala Ala Leu Met Asn Gly Gln Tyr Leu 235 240 245 250	1011
TGT GTT CAT GGT GGT ATA TCT CCC GAG TTA AAC TCT TTA CAG GAC ATT Cys Val His Gly Gly Ile Ser Pro Glu Leu Asn Ser Leu Gln Asp Ile 255 260 265	1059
AAC AAC CTA AAT AGA TTC AGG GAG ATT CCC TCT CAT GGC CTG ATG TGT Asn Asn Leu Asn Arg Phe Arg Glu Ile Pro Ser His Gly Leu Met Cys 270 275 280	1107
GAT CTG TTG TGG GCT GAC CCG ATT GAA GAG TAC GAC GAA GTC TTG GAT Asp Leu Leu Trp Ala Asp Pro Ile Glu Glu Tyr Asp Glu Val Leu Asp 285 290 295	1155
AAA GAC TTG ACT GAG GAA GAC ATA GTG AAC TCC AAA ACC ATG GTT CCT Lys Asp Leu Thr Glu Glu Asp Ile Val Asn Ser Lys Thr Met Val Pro 300 305 310	1203
CAT CAT GGC AAG ATG GCA CCT TCA AGG GAT ATG TTT GTC CCA AAC TCA His His Gly Lys Met Ala Pro Ser Arg Asp Met Phe Val Pro Asn Ser 315 320 325 330	1251
GTA AGG GGC TGT TCA TAT GCC TTC ACG TAT CGT GCT GCG TGC CAT TTT Val Arg Gly Cys Ser Tyr Ala Phe Thr Tyr Arg Ala Ala Cys His Phe 335 340 345	1299
CTG CAA GAG ACT GGC CTG TTG TCC ATC ATC AGG GCA CAC GAG GCT CAA Leu Gln Glu Thr Gly Leu Leu Ser Ile Ile Arg Ala His Glu Ala Gln 350 355 360	1347
GAC GCT GGT TAT AGA ATG TAC AAA AAT ACC AAG ACT TTG GGC TTT CCC Asp Ala Gly Tyr Arg Met Tyr Lys Asn Thr Lys Thr Leu Gly Phe Pro 365 370 375	1395
TCT CTT TTG ACC CTT TTC AGT GCG CCT AAC TAC TTG GAC ACC TAC AAT Ser Leu Leu Thr Leu Phe Ser Ala Pro Asn Tyr Leu Asp Thr Tyr Asn 380 385 390	1443
AAT AAG GCT GCC ATA TTG AAA TAC GAA AAT AAT GTT ATG AAT ATC AGA Asn Lys Ala Ala Ile Leu Lys Tyr Glu Asn Asn Val Met Asn Ile Arg 395 400 405 410	1491
CAA TTC AAC ATG ACT CCA CAC CCC TAT TGG TTA CCA GAT TTC ATG GAC Gln Phe Asn Met Thr Pro His Pro Tyr Trp Leu Pro Asp Phe Met Asp 415 420 425	1539
GTT TTC ACG TGG TCC TTG CCA TTT GTT GGT GAA AAA GTT ACA GAG ATG Val Phe Thr Trp Ser Leu Pro Phe Val Gly Glu Lys Val Thr Glu Met 430 435 440	1587
CTT GTC GCA ATT CTA AAC ATC TGT ACT GAA GAT GAG CTG GAA AAC GAC Leu Val Ala Ile Leu Asn Ile Cys Thr Glu Asp Glu Leu Glu Asn Asp 445 450 455	1635
ACC CCC GTC ATT GAA GAA TTA GTT GGT ACC GAT AAA AAA TTG CCA CAA Thr Pro Val Ile Glu Glu Leu Val Gly Thr Asp Lys Lys Leu Pro Gln 460 465 470	1683



GCT GGT AAG TCG GAA GCA ACT CCA CAA CCA GCC ACT TCG GCG TCG CCT 1731  
 Ala Gly Lys Ser Glu Ala Thr Pro Gln Pro Ala Thr Ser Ala Ser Pro  
 475 480 485 490

AAA CAT GCT TCC ATT TTA GAT GAC GAA CAT CGA AGG 1767  
 Lys His Ala Ser Ile Leu Asp Asp Glu His Arg Arg  
 495 500

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 502 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Ser Ser Asp Ala Ile Arg Asn Thr Glu Gln Ile Asn Ala Ala Ile  
 1 5 10 15  
 Lys Ile Ile Glu Asn Lys Thr Glu Arg Pro Gln Ser Ser Thr Thr Pro  
 20 25 30  
 Ile Asp Ser Lys Ala Ser Thr Val Ala Ala Ala Asn Ser Thr Ala Thr  
 35 40 45  
 Glu Thr Ser Arg Asp Leu Thr Gln Tyr Thr Leu Asp Asp Gly Arg Val  
 50 55 60  
 Val Ser Thr Asn Arg Arg Ile Met Asn Lys Val Pro Ala Ile Thr Ser  
 65 70 75 80  
 His Val Pro Thr Asp Glu Glu Leu Phe Gln Pro Asn Gly Ile Pro Arg  
 85 90 95  
 His Glu Phe Leu Arg Asp His Phe Lys Arg Glu Gly Lys Leu Ser Ala  
 100 105 110  
 Ala Gln Ala Ala Arg Ile Val Thr Leu Ala Thr Glu Leu Phe Ser Lys  
 115 120 125  
 Glu Pro Asn Leu Ile Ser Val Pro Ala Pro Ile Thr Val Cys Gly Asp  
 130 135 140  
 Ile His Gly Gln Tyr Phe Asp Leu Leu Lys Leu Phe Glu Val Gly Gly  
 145 150 155 160  
 Asp Pro Ala Thr Thr Ser Tyr Leu Phe Leu Gly Asp Tyr Val Asp Arg  
 165 170 175  
 Gly Ser Phe Ser Phe Glu Cys Leu Ile Tyr Leu Tyr Ser Leu Lys Leu  
 180 185 190  
 Asn Phe Asn Asp His Phe Trp Leu Leu Arg Gly Asn His Glu Cys Lys  
 195 200 205  
 His Leu Thr Ser Tyr Phe Thr Phe Lys Asn Glu Met Leu His Lys Tyr  
 210 215 220  
 Asn Leu Asp Ile Tyr Glu Lys Cys Cys Glu Ser Phe Asn Asn Leu Pro  
 225 230 235 240  
 Leu Ala Ala Leu Met Asn Gly Gln Tyr Leu Cys Val His Gly Gly Ile  
 245 250 255

Ser Pro Glu Leu Asn Ser Leu Gln Asp Ile Asn Asn Leu Asn Arg Phe  
 260 265 270  
 Arg Glu Ile Pro Ser His Gly Leu Met Cys Asp Leu Leu Trp Ala Asp  
 275 280 285  
 Pro Ile Glu Glu Tyr Asp Glu Val Leu Asp Lys Asp Leu Thr Glu Glu  
 290 295 300  
 Asp Ile Val Asn Ser Lys Thr Met Val Pro His His Gly Lys Met Ala  
 305 310 315 320  
 Pro Ser Arg Asp Met Phe Val Pro Asn Ser Val Arg Gly Cys Ser Tyr  
 325 330 335  
 Ala Phe Thr Tyr Arg Ala Ala Cys His Phe Leu Gln Glu Thr Gly Leu  
 340 345 350  
 Leu Ser Ile Ile Arg Ala His Glu Ala Gln Asp Ala Gly Tyr Arg Met  
 355 360 365  
 Tyr Lys Asn Thr Lys Thr Leu Gly Phe Pro Ser Leu Leu Thr Leu Phe  
 370 375 380  
 Ser Ala Pro Asn Tyr Leu Asp Thr Tyr Asn Asn Lys Ala Ala Ile Leu  
 385 390 395 400  
 Lys Tyr Glu Asn Asn Val Met Asn Ile Arg Gln Phe Asn Met Thr Pro  
 405 410 415  
 His Pro Tyr Trp Leu Pro Asp Phe Met Asp Val Phe Thr Trp Ser Leu  
 420 425 430  
 Pro Phe Val Gly Glu Lys Val Thr Glu Met Leu Val Ala Ile Leu Asn  
 435 440 445  
 Ile Cys Thr Glu Asp Glu Leu Glu Asn Asp Thr Pro Val Ile Glu Glu  
 450 455 460  
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 465 470 475 480  
 Thr Pro Gln Pro Ala Thr Ser Ala Ser Pro Lys His Ala Ser Ile Leu  
 485 490 495  
 Asp Asp Glu His Arg Arg  
 500

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: G4-PCR-A

73

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCCCTATCGT GCACTCACCG ACGC

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(2) INFORMATION FOR SEQ ID NO:23:

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  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
  - (C) INDIVIDUAL ISOLATE: G4-PCR-B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTGAAGGCCC TACTGAGCCA GGAG

24

## IT IS CLAIMED:

1. A polypeptide composition comprising a polypeptide effective to enhance immunosuppressive effects of a calcineurin-targeted immunosuppressant by potentiating an interaction of an immunophilin with calcineurin.  
5
2. A composition of claim 1, wherein the polypeptide composition contains a calcineurin interacting (CNI) polypeptide.
- 10 3. A composition of claim 2, wherein the polypeptide composition contains a polypeptide having an amino acid sequence selected from the group consisting of sequences represented by SEQ ID NO:2 and SEQ ID NO:5.
4. A composition of claim 2, wherein the polypeptide has an amino acid sequence of  
15 between 15 and 915 amino acids in length.
5. A composition of claim 2, wherein the polypeptide composition contains a polypeptide having an amino acid sequence comprising the c-terminal 306 amino acids of a CNI protein.  
20
6. An isolated nucleic acid having a sequence encoding a polypeptide of any of claims 1-5.
7. A nucleic acid of claim 6, wherein the nucleic acid has a sequence selected from  
25 the group consisting of nucleic acid sequences represented by SEQ ID NO:3 and SEQ ID NO:6.
8. A method of identifying a small molecule immunosuppressant compound, comprising  
30 constructing a cell-based two hybrid protein-protein interaction assay, wherein one of two fusion hybrid proteins in a cell contains an (A) subunit of calcineurin, and the other of two fusion hybrid proteins contains a CNI polypeptide,  
contacting the cell with a small molecule, and

identifying the small molecule as an immunosuppressant if the molecule potentiates an interaction between the two hybrid proteins.

9. A method of claim 8, wherein the cell is a yeast cell.

5

10. A method of claim 8, wherein one of the two fusion hybrid proteins contains a GAL4 activation domain and the other of two fusion hybrid proteins contains a GAL4 binding domain.

10

11. A method of claim 8, wherein the subunit of calcineurin is selected from the group consisting of yeast CNA1 and yeast CNA2.

12. A method of claim 8, wherein the subunit of calcineurin is an "A" subunit of human calcineurin.

15

13. A method of claim 8, wherein the CNI polypeptide is yeast CNI polypeptide.

14. A method of claim 8, wherein the CNI polypeptide is yeast CN1c polypeptide.

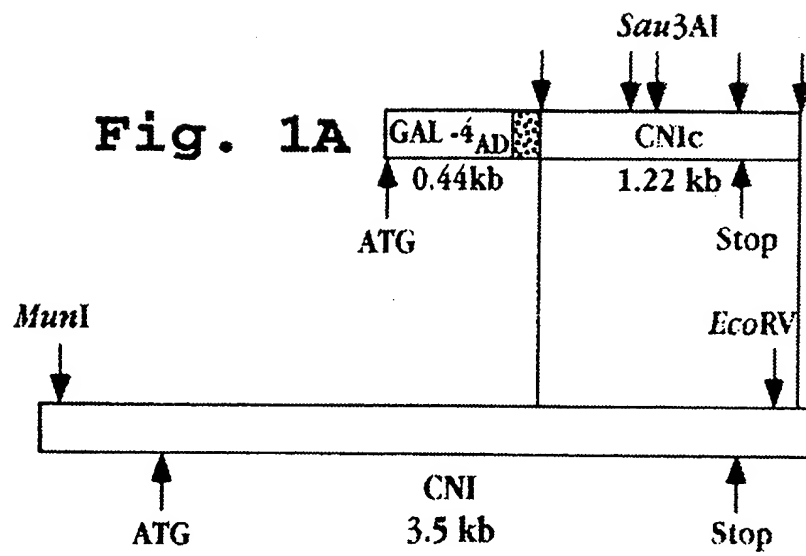
20

15. A method of claim 8, wherein the cell is further modified to cause overexpression of a "B" subunit of calcineurin by said cell.

16. A yeast cell carrying a mutation in the naturally-occurring genomic copy of a gene encoding calcineurin-interacting polypeptide, where said mutation prevents expression of a functional calcineurin-interacting polypeptide from said genomic copy.

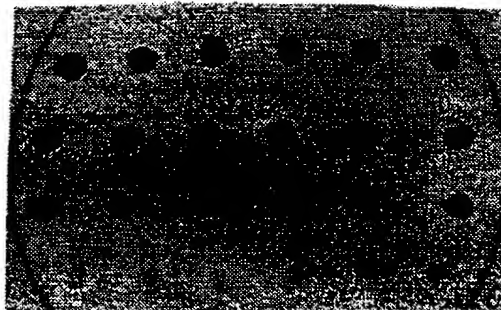
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1/11

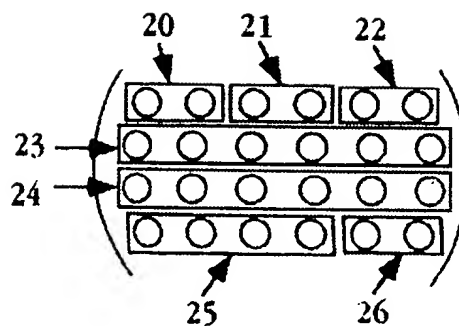


**Fig. 1B**

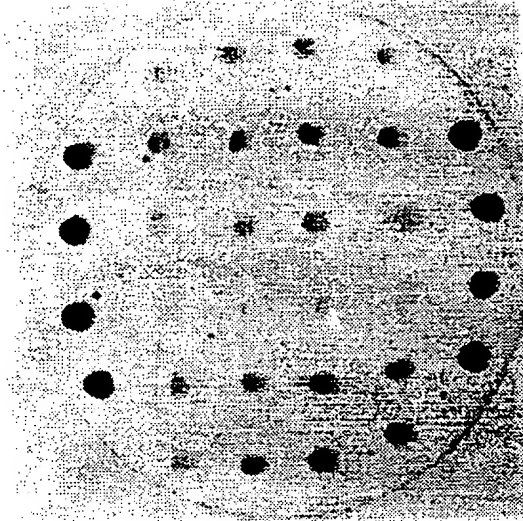
**Fig. 2A**



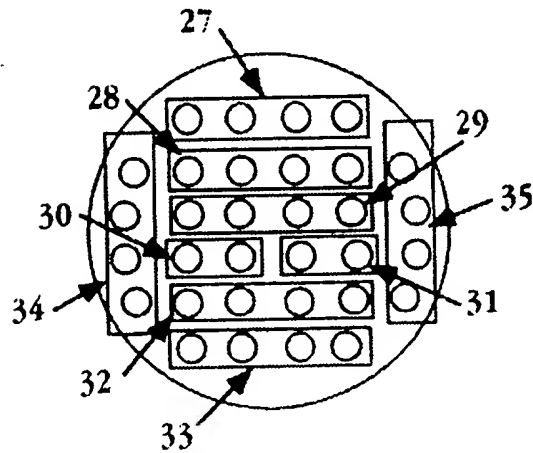
**Fig. 2B**



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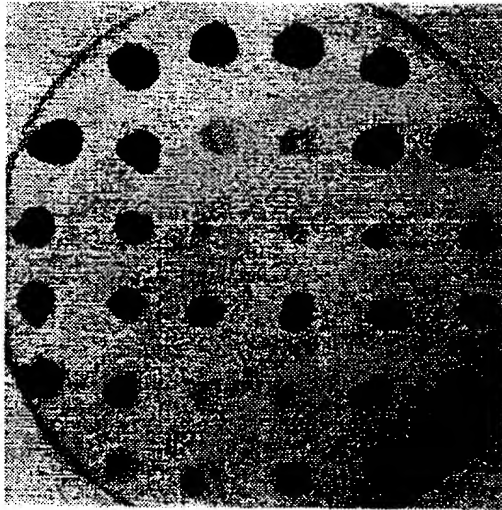
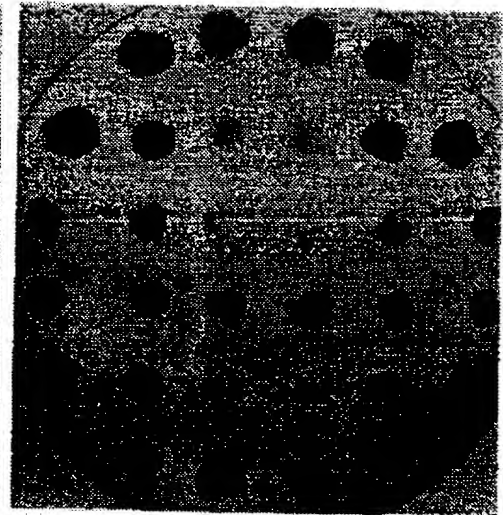
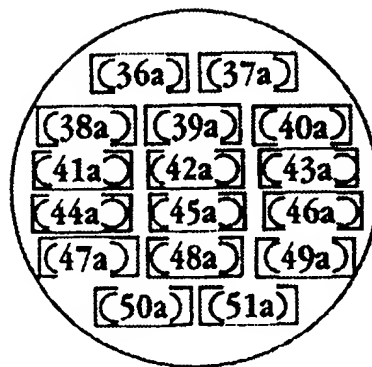
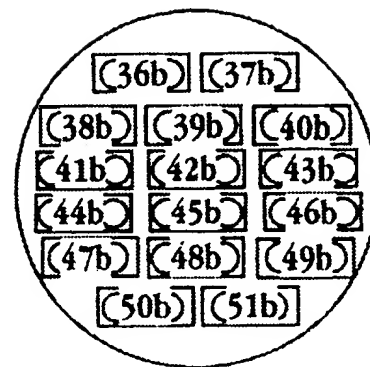


**Fig. 3A**



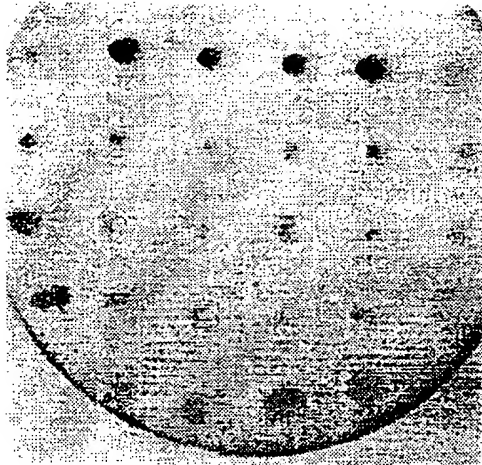
**Fig. 3B**

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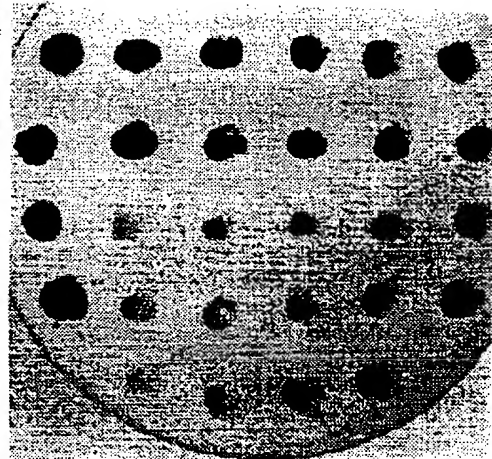
**Fig. 4A****Fig. 4C****Fig. 4B****Fig. 4D**



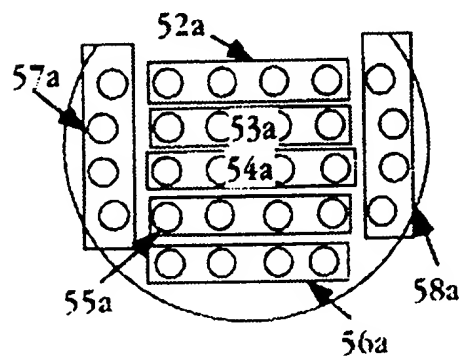
4/11



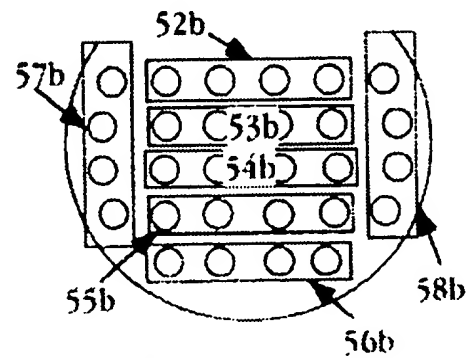
**Fig. 5A**



**Fig. 5C**



**Fig. 5B**



**Fig. 5D**

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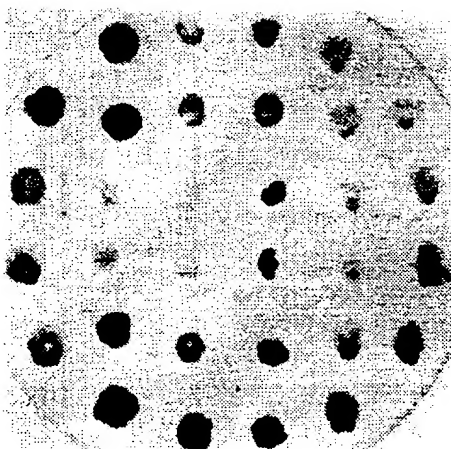


Fig. 6E

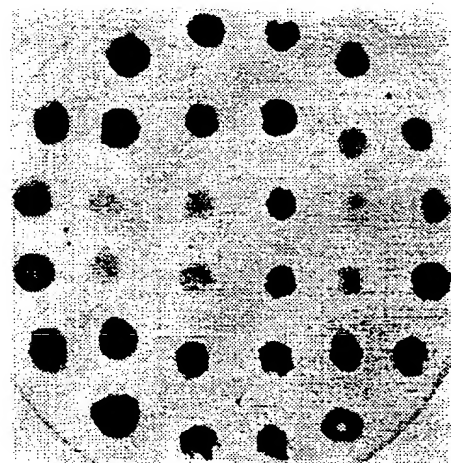


Fig. 6C

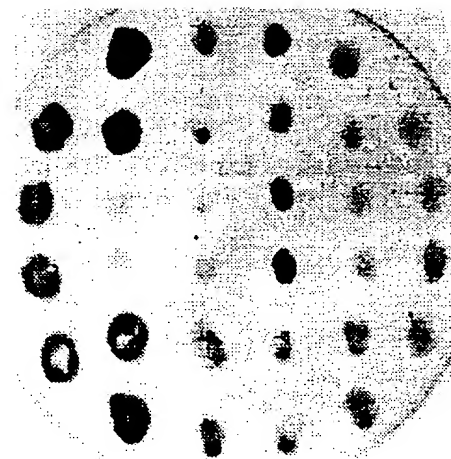


Fig. 6A

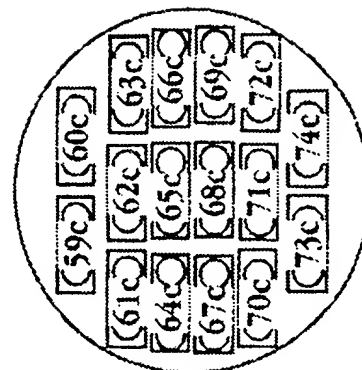


Fig. 6F

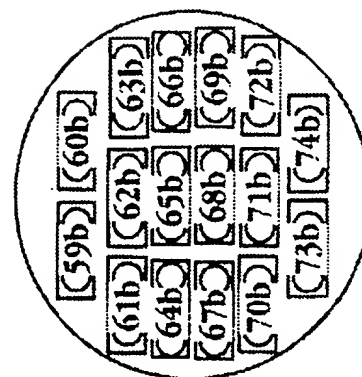


Fig. 6D

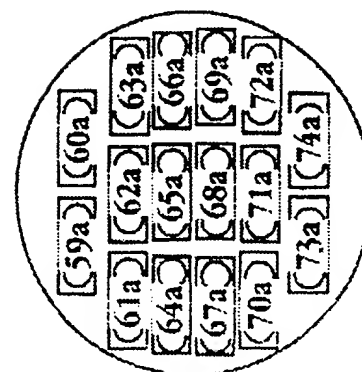


Fig. 6B

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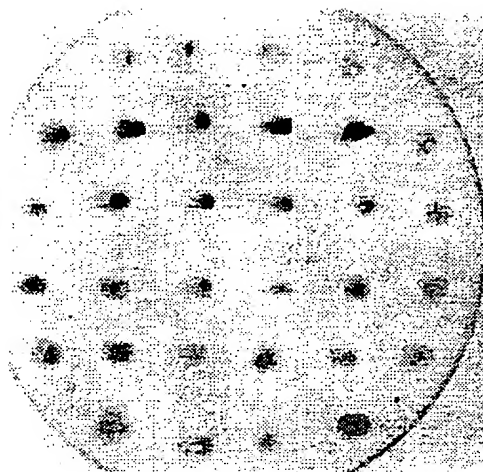


Fig. 7A

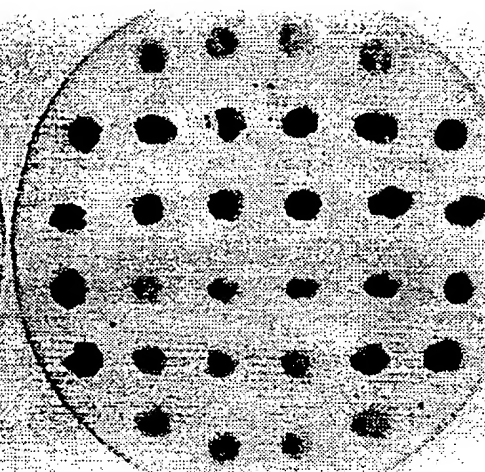


Fig. 7C

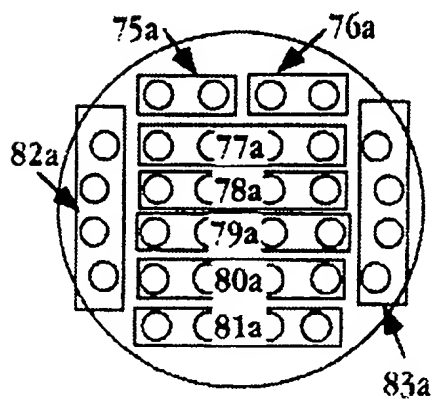


Fig. 7B

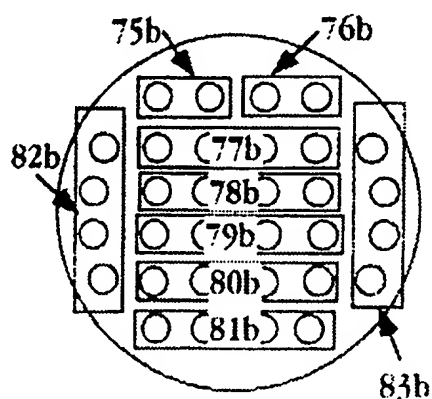
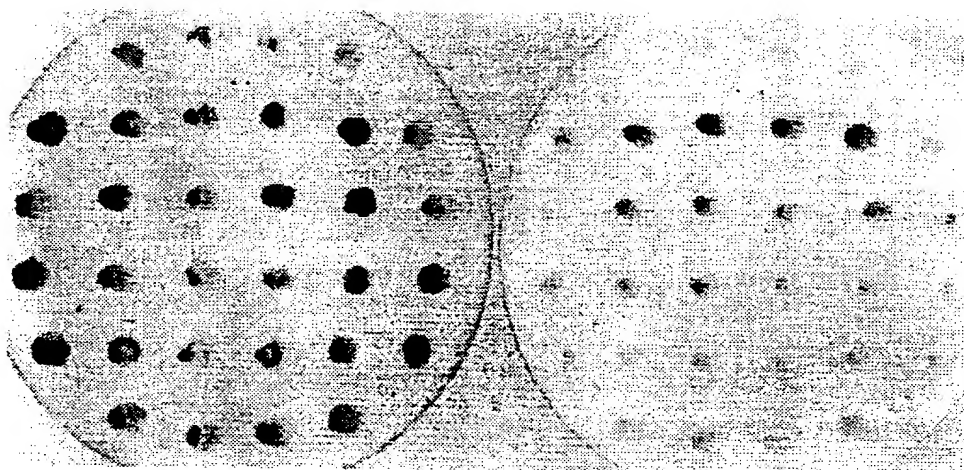
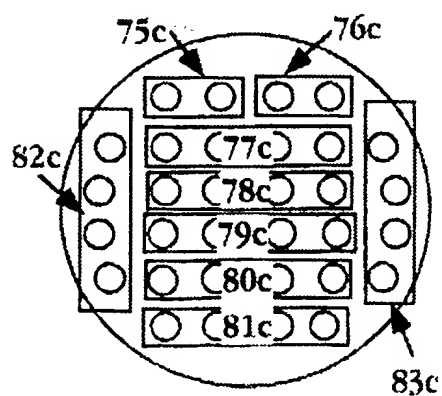
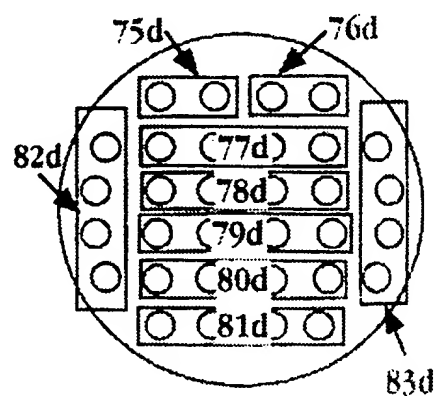
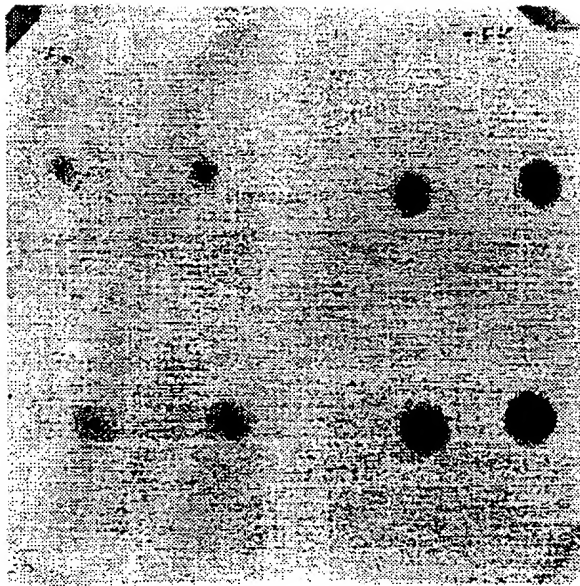
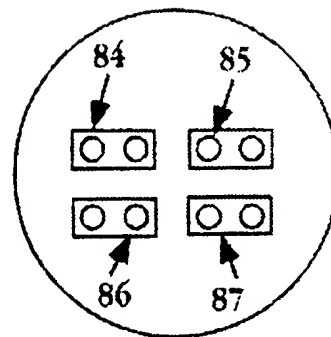
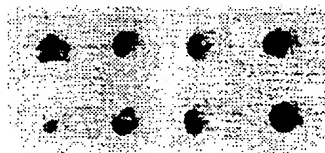
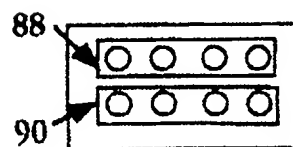
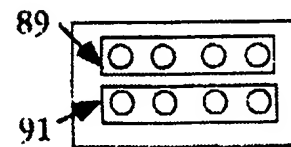


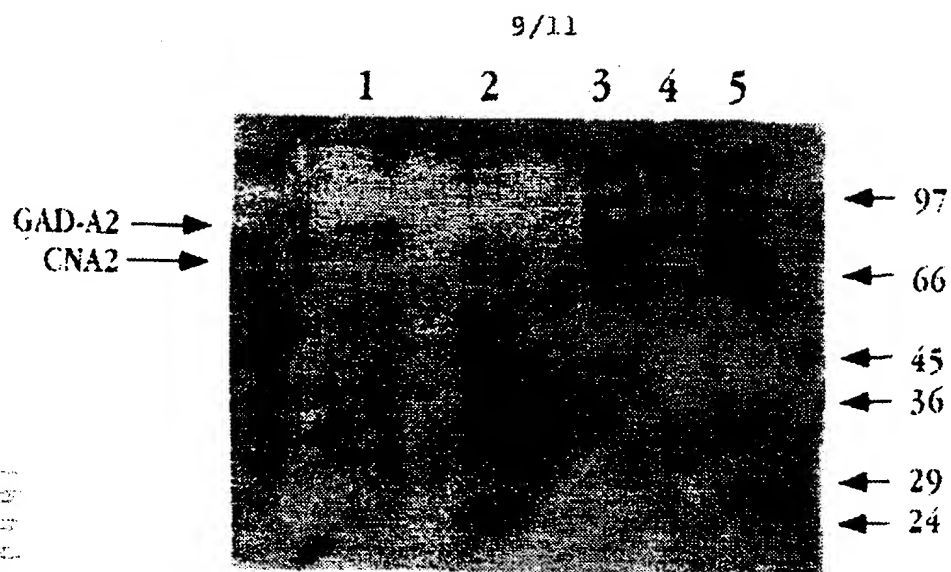
Fig. 7D

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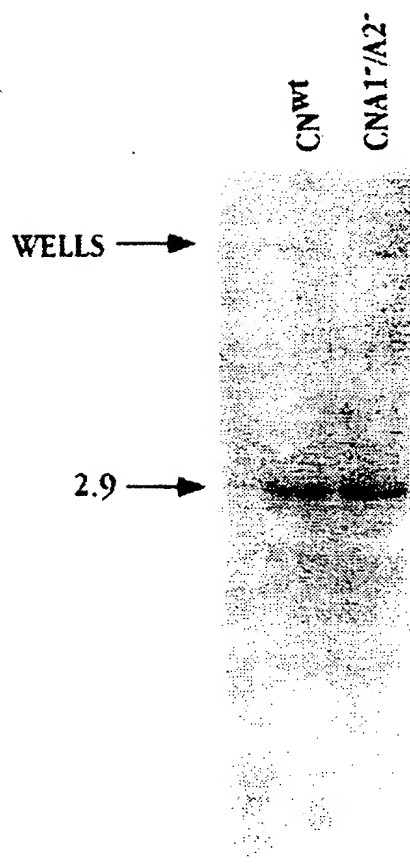
**Fig. 7E****Fig. 7G****Fig. 7F****Fig. 7H**

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**Fig. 8A****Fig. 8B****Fig. 9A****Fig. 9C****Fig. 9B****Fig. 9D**



**Fig. 10**



**Fig. 11**

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```

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Fig. 12

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**Fig. 13**



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/13580

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/31 C07K14/395 G01N33/68 C12N15/62 C12Q1/00  
 C12N1/16 //(C12N1/16,C12R1:865)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Swissprot Database entry YK01_YEAST Accession number P36117; June 01, 1994 Duesterhoeft A. et al.: 'Hypothetical 102.5 kD protein in YPT52-GCN3 intergenic region.'	1-5
X	Emfun Database entry Scykr021w Accession number Z28246; May 10, 1994 Duesterhoeft A. et al.: 'S. cerevisiae chromosome XI reading frame ORF YKR021w.'	6,7
P,X	MOLECULAR BIOLOGY OF THE CELL, 5 (SUPPL.). 1994. 141A., HUANG L ET AL 'A novel protein that interacts with calcineurin in vivo' see abstract 818	1,2,6, 8-15
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

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- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search

21 March 1996

Date of mailing of the international search report

02.04.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+ 31-70) 340-3016

Authorized officer

Espen, J

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/13580

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CURR BIOL, 2 (1). 1992. 18-20., CYERT M S 'IMMUNOSUPPRESSANTS HIT THE TARGET' see the whole document -----</p>	